THE KINETICS OF DARK ADAPTATION.

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I.

Nature of Contents.

1. Purpose.—Many photosensitive animals acquire in the dark an increased sensibility to light. A quantitative description of this process of dark adaptation is limited, however, to very few animals. For vertebrates, there are the numerous and accurate measurements with man (Piper, 1903; Nagel, 1911; Hecht, 1921–22); the few observations with the chick (Honigmann, 1921); and the measurements with the tadpole (Obreshkove, 1921); while for invertebrates there are only the data with the clam, Mya arenaria (Hecht, 1918–19 b). Additions to this meager collection of data are obviously desirable. The first purpose of the present paper is to present measurements of the dark adaptation of two additional invertebrates: the lamellibranch, Pholas dactylus; and the ascidian, Ciona intestinalis.

The original study of the dark adaptation of Mya (Hecht, 1918–19 b) contained an analysis of the process in terms of a photosensory mechanism. Since then, much has been learned about the nature of the processes involved, and as a result certain modifications in the details of the theoretical treatment have become necessary (Hecht, 1922–23). The second purpose of this paper is to present an analysis of the data of dark adaptation which is in keeping with present knowledge.

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† The experiments here recorded were made at the Zoological Station, Naples, during my tenure of the Jacques Loeb Memorial Table. I wish to express my appreciation of the many kindnesses shown me during my stay in Naples by the Director of the Zoological Station, Dr. Reinhard Dohrn.
The organisms whose dark adaptation has been measured may be divided into two groups depending on the nature of their photosensitive structure, and on the method of making the measurements. In man and the chick the light sensitive system is a complicated vertebrate eye. Dark adaptation in these animals has been measured by finding the illumination intensity just perceptible to the eye during different moments of the stay in the dark. Thus of the two factors which control the photochemical effect of a given light, the time of exposure is held constant while the intensity is varied. However, in the other animals mentioned the photoreceptors are superficially located and are diffuse. In *Mya* and *Pholas* they are on the siphons and on the exposed parts of the mantle (Dubois, 1892; Wenrich, 1916); in *Ciona* they are in a small area between the siphons (Hecht, 1918–19 a); and in the tadpole they are in the skin (Obreshkove, 1921). With these animals dark adaptation is measured by finding the exposure required to elicit a response to a given illumination. Time is thus the variable while the intensity is constant.

The principles underlying the theoretical analysis of dark adaptation is the same in these two groups of animals (Hecht, 1919–20). But because of the difference in the method of securing the data for the two classes it is more convenient to present them separately. I shall therefore consider here only the second group. The third purpose of the present paper is to complete the analysis of this class of animals by including a study of Obreshkove's data of the dark adaptation of the tadpole.

It might appear desirable, in order to render this study complete, to collect here the bits of information about dark adaptation which are scattered in the voluminous literature of the sensitivity of animals to light. However, such a course would not be profitable, because this material consists mostly of the mere statement that a given animal can become dark adapted, coupled only occasionally with a numerical datum giving the order of magnitude of this effect (e.g. Hess, 1910). An apparent exception might seem to be the work of Folger (1924–25) who, under the heading of dark adaptation records a few measurements of the recovery of *Ameba* from stimulation by light. Folger has very clearly shown, however, that most of this recovery can occur in the light as well as in the dark. The process is, therefore, not dark
adaptation; and is not relevant to the present study dealing with the quantitative aspects of this process.

2. Definitions.—Folger's paper calls for comment here, because of the unexpected meaning which he has attached to the terms dark adaptation and light adaptation.

"An organism is said to be dark adapted when it will respond to a sudden increase in the intensity of illumination. The converse of dark adaptation is light adaptation. This is brought about by exposure to light. An animal is said to be light adapted when from a lack of dark adaptation it fails to respond to a sudden increase in illumination."

According to Folger's definition of dark adaptation, the human eye, or *Mya*, or *Ciona* are dark adapted in broad daylight, since they all respond to a sudden increase in illumination under such conditions. Similarly according to Folger, none of these organisms can become light adapted, since there are no light conditions known under which they do not respond to a sudden increase in illumination if of sufficient magnitude. It is therefore to be regretted that an erroneous and confused connotation has been applied to such terms as dark and light adaptation which have always had a precise and accepted meaning.

Aubert (1865) introduced the term adaptation into physiology when he recorded the first measurements of the dark adaptation of the eye. Since then there has been no misunderstanding of its meaning. A detailed treatment of it has been given by Nagel (1911). This is too long to be quoted here; I shall therefore summarize it briefly in a form applicable to the present situation. A dark adapted animal is one whose sensitivity to light has reached a constant value as the result of a sustained stay in the dark. A light adapted animal is one whose sensitivity to light has reached a constant value as the

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1 Folger (1924–25, p. 279).
2 This confusion is only one of the many to be found in Folger's paper,—a situation which may be best illustrated perhaps by quoting one of the major conclusions of the work (p. 290). Folger is concerned with the fact that "A mechanical shock exerts a distinct effect upon the reaction to light." "This indicates that increase in illumination and mechanical shock produce the same changes in Amoeba, that mechanical stimulation and photic stimulation are fundamentally the same. If this is true, it is evident, since mechanical stimulation is not photochemical, that photic stimulation cannot be photochemical."
result of a sustained exposure to a given intensity of illumination. The process of reaching these two states is that of dark adaptation and of light adaptation, respectively. These definitions might be supposed to be self-evident. The terms which they describe will therefore be used in their accepted sense in the present paper.

II.

Experiments with Pholas dactylus.

1. General Properties of Sensibility.—Pholas is an animal whose photosensory behavior is similar to that of Ciona and Mya. Its resemblance to Mya in particular is so extraordinary that in a short time it is possible to demonstrate in a qualitative way the existence of all the interrelations which have been quantitatively established in Mya (Hecht, 1925). The following are some of these properties; they are recorded here so that the study of the dark adaptation of Pholas may be intelligible.

(a) Pholas is very sensitive to light, and responds to it by a vigorous retraction of its extended siphon. The time from the beginning of the exposure to the beginning of the retraction was called the "latent time" by Dubois (1892) who first worked with this animal. In conformity with the less accurate but more usual practice it will be referred to as the "reaction time."

(b) This reaction time is not a simple period. As in the case of Ciona and Mya, it is composed of two parts, an exposure period and a latent period. The first is occupied by the necessary exposure to light. It varies with the intensity and can be very short with high intensities. The second, or latent period, occupies most of the reaction time. During the latent period the animal may remain in the dark and still respond after the usual reaction time. Thus a reaction time of 2 seconds to an illumination of 500 meter candles is composed almost entirely of latent period, because the actual exposure to light need be only 0.02 second.

(c) Up to a certain limit of exposure the latent period varies inversely with the duration of the exposure.

(d) Temperature has almost no influence on the exposure period, as is to be expected if the exposure is concerned with a photochemical
reaction. The latent period, however, is definitely influenced by temperature, in a manner similar to most "dark" reactions.

(e) *Pholas* comes into sensory equilibrium with any illumination to which it is exposed continuously. Its first act on being illuminated is to retract its siphon. In a few seconds, however, it extends the siphon and appears to be unstimulated by the light. Its sensitivity is now much less than before, because the intensity of illumination has to be considerably augmented in order to cause *Pholas* to respond again.

(f) Its original sensibility may be restored by placing the animal in the dark. This is a fairly slow process in *Pholas*, as will be apparent presently.

Dubois (1892) failed to note many of these properties of the sensory process, because of his interest in the contraction of the siphon. He laid great stress on the fact that the siphon response may be recorded graphically, and most of his work was concerned with the form of the siphon contraction under different conditions of stimulation. To Dubois is due the important point that the photosensory process and the resulting retraction response are essentially independent of the rest of the animal, because he showed that a detached siphon retains its sensibility to light and its capacity to contract for several days after removal from the rest of the animal.

2. Dark Adaptation.—If after exposure to strong light, *Pholas* is placed in the dark, its reaction time to a light of constant intensity decreases steadily until it reaches a constant minimum characteristic of its response to that intensity. The course of this dark adaptation has been investigated in the following manner. An animal is exposed to an illumination of 10,000 meter candles. After the response, it almost at once expands its siphon. 2 or 3 minutes seem to be sufficient for light adaptation, but 7 minutes are allowed in these experiments. The animal is then placed in the dark and during the next 2 hours its reaction time to an illumination of 30 meter candles is measured three times. Several hours later the animal is again light adapted as before; it is placed in the dark and its reaction time to 30 meter candles is measured at such times that all together there are secured six values of the reaction time at half hour intervals during dark adaptation. The measurements are made in two series instead of one, so as to
allow an hour between successive exposures. In this way the progress of dark adaptation is disturbed as little as possible by the short exposures to light needed for making the measurement.

During the stay in the dark the animal in its rectangular glass dish is kept in a water bath so as to maintain the temperature constant. The source of light is properly screened in a box, and water filters are interposed to reduce the heat to a minimum. Though negligible during a measurement, this factor must be controlled during the 7 minutes light adaptation when the temperature is kept constant by the addition of cold sea water to the dish.

### TABLE I.

**Dark Adaptation of Pholas. 18 Animals. Temperature, 16.5°C. Latent Period, 1.00 Second.**  
$k = 0.0143; a = 0.730$.  

<table>
<thead>
<tr>
<th>Time in dark $t$ (min.)</th>
<th>Reaction time $r$ (sec.)</th>
<th>Observed</th>
<th>$r = \frac{93.8}{t + 1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.95</td>
<td></td>
<td>2.95</td>
</tr>
<tr>
<td>30</td>
<td>2.23</td>
<td></td>
<td>2.25</td>
</tr>
<tr>
<td>60</td>
<td>1.81</td>
<td></td>
<td>1.81</td>
</tr>
<tr>
<td>90</td>
<td>1.65</td>
<td></td>
<td>1.60</td>
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<tr>
<td>120</td>
<td>1.46</td>
<td></td>
<td>1.48</td>
</tr>
<tr>
<td>150</td>
<td>1.40</td>
<td></td>
<td>1.40</td>
</tr>
</tbody>
</table>

With Pholas I measured the dark adaptation of 18 animals. The data secured are given in Table I, and graphically in Fig. 1. It is apparent that the process is regular, and at this temperature takes more than 3 hours to become complete.

3. **Theoretical Analysis of Dark Adaptation.**—The reaction time of Pholas to light is an interval occupied by several processes which combine to produce a constant effect, namely a siphon retraction. The algebraic sum of the velocities of these separate processes is represented by the reciprocal of the reaction time. Only two of these processes, the photochemical and the latent period, need be considered, since the time occupied by conduction and central nervous activity is negligible in stop-watch measurements and may be included in the latent period. The data therefore show that the sum of the velocities of the two major processes increases during dark adaptation.
The work with *Mya* (Hecht, 1922–23; 1923–24) has shown that to cause a given photosensory response a definite and constant amount of photochemical decomposition must be produced by the incident light. Let us assume that this holds during the dark adaptation of *Pholas*, and therefore that the photochemical effect during each re-

![Graph](https://i.imgur.com/3Q5z5Q5.png)

**Fig. 1.** Dark adaptation of *Pholas*. Each point is the average of 18 measurements, one with each of 18 animals. The first, third, and fifth points on the curve for each animal were made during one run of dark adaptation, while the other three points were made during another run several hours later. The curve is calculated in terms of a bimolecular reaction.

action time in Table I is constant. This is the only assumption which has to be made in order to understand dark adaptation.

It is already well known that the velocity of the latent period is directly proportional to the magnitude of photochemical effect produced during the exposure (Hecht, 1918–19 c; 1925–27). If the photo-
chemical effect be assumed constant, it follows that the velocity of the latent period is also constant. The total velocity of the reaction time processes is thus composed of two velocities, one of which is constant. Any increase in total velocity must therefore represent an increase in the velocity of the primary photochemical process only; and hence the curve in Fig. 1 represents the changes in the velocity of the photochemical reaction during dark adaptation.

Since the intensity of the measuring light is constant, the change in velocity of the primary photochemical reaction is very likely due to a change in the concentration of sensitive substances accumulating during dark adaptation. On obvious photochemical grounds, it may be supposed that the velocity of the reaction is proportional to the concentration of photosensitive substance in the sense cells. It then follows that the concentration of sensitive material increases in conformity with the shape of the experimental curve of dark adaptation. When the form of the curve in Fig. 1 is investigated it is found to be that of a bimolecular reaction isotherm

\[ k_1 = \frac{x}{t (a - x)} \tag{1} \]

where \( k \) is the velocity constant; \( t \) the time of dark adaptation; \( x \) the concentration of photosensitive material already formed; and \( a - x \)

This does not mean that the duration of the latent period is necessarily constant during the large changes in reaction time associated with dark adaptation. In fact, it is known that the latent period is not quite constant under such conditions (Hecht, 1922–23, p. 573). What is constant is the average velocity of the latent period process. It has been shown experimentally (Hecht, 1918–19; 1925–27) that the reaction underlying the latent period depends for its progress on the products formed by the photochemical reaction during the exposure. If the exposure is long, the latent period reaction begins as soon as some photochemical products are formed, and increases in velocity as these increase in concentration. The average velocity of the latent period process is then constant, though its actual duration may vary slightly. If, however, the necessary exposure is short in comparison with the latent period the former may be considered instantaneous, and therefore both the velocity of the latent period and its duration will be constant.

\[4\] This proportionality between concentration of sensitive material and velocity of reaction is a first approximation, and holds strictly in an irreversible reaction only. However, since the analysis in terms of it is descriptive of the data, it is retained in its simple form without the added term for the reversible reaction.
the concentration still to be formed. The third column in Table I gives the calculated values of the reaction time on which the theoretical curve in Fig. 1 is based.

It is necessary to describe in detail the method of calculating the terms in the above equation in order to show how they are derived from the experimental data of Table I. The limits between which the velocity of the reaction time processes can vary are the minimum velocity at the first moment of dark adaptation, and the maximum possible velocity as given by the velocity of the latent period alone. The range between these two limits represents the possible variation in the speed of the photochemical reaction, and corresponds to the

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Reaction time</th>
<th>Latent period</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11</td>
<td>1.47</td>
<td>1.36</td>
</tr>
<tr>
<td>0.20</td>
<td>1.37</td>
<td>1.17</td>
</tr>
<tr>
<td>0.28</td>
<td>1.28</td>
<td>1.00</td>
</tr>
<tr>
<td>0.35</td>
<td>1.27</td>
<td>(1.00)</td>
</tr>
<tr>
<td>0.41</td>
<td>1.28</td>
<td>(1.00)</td>
</tr>
</tbody>
</table>

total change $a$ in concentration of sensitive material $S$. If $x$ is the concentration of $S$ at the moment $t$, then $a - x$ is the concentration of $S$ still to be formed, and corresponds to the difference between the maximum velocity and the velocity at the moment $t$; i.e., the difference between the reciprocal of the latent period and the reciprocal of the reaction time at the moment $t$.

The reaction time values are of course the measurements in Table I. The latent period is determined by a separate experiment with the same animals. An animal is stimulated at hourly intervals by a series of graded exposures, and the reaction time measured. Table II gives the data for 16 of the animals used in Table I. It is apparent that the smallest exposure giving the minimum reaction time of 1.28 seconds is 0.28 seconds. The minimum reaction time minus the minimum exposure is obviously the latent period, and is equal to 1.00 second.
The value $a - x$ at the moment $t$ is thus a difference between the reciprocals of two experimentally determined magnitudes. If now $\frac{1}{a - x}$ is plotted against $t$ the points should lie on a straight line provided the reaction is bimolecular. This is because equation (1) can be converted into the form of a straight line

$$\frac{1}{a - x} = kt + \frac{1}{a}$$

(2)
If we call $r$ the duration of the reaction time, and $p$ that of the latent period, then $a - x$ will be represented by $1/p - 1/r$, and $\frac{1}{a - x}$ by $\frac{r}{r - p}$ according to the above reasoning. Fig. 2 shows that if $\frac{1}{a - x}$ or its equivalent $\frac{rp}{r - p}$ is plotted in this way, the data conform to equation (2). The tangent of the line is $k$, the reciprocal of its intercept on the ordinate axis is $a$. The significance of this graphic mode of computation is that the values of $k$ and $a$ are both found directly from the experimental data, and are not arbitrarily assumed in the calculations.

4. Interpretation.—In making the above analysis no assumption was made with regard to the way in which the sensitive material accumulates in the sense cells during dark adaptation. The agreement between the kinetics of a simple bimolecular reaction and the course of dark adaptation permits one to draw the conclusion that the process is chemical in nature. If the kinetics of dark adaptation had followed a monomolecular isotherm this interpretation would have been doubtful because under certain conditions the course of a diffusion process may also be described that way. As it is one must suppose that two substances at least are concerned in the formation of the sensitive material, and that these two substances combine in a chemical manner.

It is apparent that the chemical nature of the process can be corroborated by studying the effect of temperature on dark adaptation. This was not done with Pholas, but has been done with Mya, and the results as given in the next section bear out the chemical interpretation of the present data.

III.

**Mya arenaria.**

1. Assumptions.—The original study with Mya contained an analysis of its dark adaptation on the basis of two assumptions. These were first, that the photochemical effect of light on the sensory system is directly proportional to the exposure; and second, that the concentration of photochemical decomposition products required for a
response is proportional to the concentration of products already present in the sense cell. In terms of them dark adaptation was shown to follow the kinetics of a bimolecular reaction.

However, both assumptions have proven inadequate for the further handling of the characteristics of the photosensory process, and have been discarded (Hecht, 1922-23). Therefore, it is proposed to show here how from the same data the same conclusion may be reached by the simpler and more plausible analysis just used with *Pholas*. This entails only one assumption: under all circumstances a definite amount of photochemical decomposition is required to produce a

<table>
<thead>
<tr>
<th>Time in dark (min.)</th>
<th>Observed (sec.)</th>
<th>Calculated in original way (sec.)</th>
<th>Calculated in present way (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.28</td>
<td>2.21</td>
<td>2.24</td>
</tr>
<tr>
<td>8</td>
<td>1.95</td>
<td>1.92</td>
<td>1.94</td>
</tr>
<tr>
<td>14</td>
<td>1.70</td>
<td>1.71</td>
<td>1.74</td>
</tr>
<tr>
<td>20</td>
<td>1.60</td>
<td>1.60</td>
<td>1.63</td>
</tr>
<tr>
<td>29</td>
<td>1.51</td>
<td>1.53</td>
<td>1.55</td>
</tr>
<tr>
<td>40</td>
<td>1.50</td>
<td>1.48</td>
<td>1.49</td>
</tr>
<tr>
<td>53</td>
<td>1.50</td>
<td>1.47</td>
<td>1.45</td>
</tr>
</tbody>
</table>


given sensory effect. Though given here as an assumption purely, its validity is attested by a good deal of experimental evidence (Hecht, 1922-23; 1923-24).

2. *Dark Adaptation.*—The data, given in Table III, are the original ones for *Mya*, and were secured by methods which are essentially those described above for *Pholas*. With the data are the values calculated in the present manner as given in the previous section with *Pholas*, and in the old manner. It is clear that the two modes of theoretical treatment give the same approximation to the experimental data. This follows from the fact first, that the curve of dark adaptation is an hyperbola, and second, that the two methods of
calculation involve the use either of a given value of the ordinates (reaction time) or of its reciprocal.

It may not be amiss to indicate precisely why the two methods give almost identical results. Let \( r_t \) be the reaction time after \( t \) minutes of dark adaptation, and \( r_0 \) its value at the first moment when \( t = 0 \). Let \( \rho \) be the latent period. Then according to the original hypothesis

\[
\begin{align*}
a &= k_1 (r_0 - \rho) \\
x &= k_1 (r_0 - r_t) \\
a - x &= k_1 (r_t - \rho)
\end{align*}
\]

where \( a \) is the total amount of sensitive material to be formed, \( x \) the amount already formed, and \( a - x \) the amount still to be formed from the precursors according to the usual equation

\[
k_1 = \frac{x}{a (a - x)}
\]

for a bimolecular process. If the corresponding values in (3), (4), and (5) are substituted in (1) it becomes

\[
k = \frac{r_0 - r_t}{(r_0 - \rho) (r_t - \rho)}
\]

which describes the behavior of the experimental data. According to the present alternative hypothesis it is

\[
\begin{align*}
a &= k_2 \left( \frac{1}{\rho} - \frac{1}{r_0} \right) \\
x &= k_2 \left( \frac{1}{r_t} - \frac{1}{r_0} \right) \\
a - x &= k_2 \left( \frac{1}{\rho} - \frac{1}{r_t} \right)
\end{align*}
\]

which describe the relations, and which give

\[
k = \frac{\frac{1}{r_t} - \frac{1}{r_0}}{\left( \frac{1}{\rho} - \frac{1}{r_0} \right) \left( \frac{1}{\rho} - \frac{1}{r_t} \right)}
\]
when substituted in equation (1). Equation (10) reduces very simply to

\[ k \rho^2 = \frac{r_e - r_t}{t(r_e - \rho)(r_t - \rho)} \]

(11)

which, since \( \rho \) is constant, is identical in form with equation (6).

The choice between the two hypotheses is therefore not so much as to which gives a better agreement with the data of dark adaptation, but as to which is more consistent with other work, and inherently perhaps more plausible.

**TABLE IV.**

Dark Adaptation of *Mya* at Different Temperatures. Data from Hecht.* 5 Animals.

<table>
<thead>
<tr>
<th>Time in dark</th>
<th>Reaction time</th>
<th></th>
<th>Time in dark</th>
<th>Reaction time</th>
<th></th>
<th>Time in dark</th>
<th>Reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>sec.</td>
<td>sec.</td>
<td>min.</td>
<td>sec.</td>
<td>sec.</td>
<td>min.</td>
<td>sec.</td>
</tr>
<tr>
<td>11</td>
<td>5.04</td>
<td>4.72</td>
<td>16.2°C.; ( k = 0.124 )</td>
<td>29.5</td>
<td>3.94</td>
<td>3.94</td>
<td>13.5</td>
</tr>
<tr>
<td>15</td>
<td>4.13</td>
<td>4.31</td>
<td>20</td>
<td>3.50</td>
<td>3.48</td>
<td>19.5</td>
<td>1.64</td>
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<tr>
<td>20</td>
<td>3.94</td>
<td>3.94</td>
<td>29.5</td>
<td>3.11</td>
<td>3.20</td>
<td>30.0</td>
<td>2.12</td>
</tr>
<tr>
<td>29.5</td>
<td>3.50</td>
<td>3.48</td>
<td>39.5</td>
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<td>54.5</td>
<td>1.90</td>
</tr>
<tr>
<td>39.5</td>
<td>3.11</td>
<td>3.20</td>
<td>55</td>
<td>2.05</td>
<td>1.98</td>
<td>40.5</td>
<td>1.90</td>
</tr>
</tbody>
</table>

\* Hecht (1918–19 b), p. 556.

3. Dark Adaptation and Temperature.—The present theoretical analysis of the data of *Mya* brings to light a quantitative relation between dark adaptation and temperature. The purpose of the original experiments with temperature was to measure the temperature coefficient of dark adaptation in order to ascertain whether the process underlying dark adaptation is chemical in nature, in this way corroborating the evidence from its kinetics. The temperature coefficient, \( Q_{10} \), for the velocity constant \( k \) confirmed this by being near 2.5. Nevertheless the values of \( k \) as derived from the original computations did not fit the Arrhenius equation relating temperature with the velocity constant of a chemical reaction (Arrhenius, 1912).
It is a significant point that recalculation of the same data in terms of the present analysis yields a series of values for the velocity constant $k$ which show an excellent agreement with the Arrhenius equation.

The experimental data for dark adaptation at three temperatures are given in Table IV. They are shown graphically in Fig. 3 according to the method of analysis described above. The corresponding calculated values are also given in Table IV, from which, as well as from Fig. 3, may be noted the adequacy of a bimolecular reaction for describing dark adaptation.

The velocity constants $k$ derived from these data are given in

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**Fig. 3.** Dark adaptation of *Mva* at three temperatures. The ordinates are $r \frac{p}{(r-p)}$ which is equivalent to $1/(a-x)$ in the equation for a bimolecular process. The velocity of the reaction as indicated by the slope of the lines increases with the temperature.
Table IV, and their relation to the temperature in Fig. 4. For purposes of graphic presentation the Arrhenius equation may be written

\[ \ln k = -\frac{\mu}{RT} + C \]

(12)

where \( k \) is the velocity constant at the absolute temperature \( T \), \( C \) is a constant of no significance here, \( R \) is the gas constant, and \( \mu \) is the critical increment or temperature characteristic (Crozier, 1924). A plot between \( \ln k \) and \( 1/T \) should be a straight line whose tangent is \( \mu \). The data yield such a relation with \( \mu = 17,400 \). In judging of the reliability of the three points through which the straight line passes, it is to be remembered that each velocity constant \( k \) is computed from at least 6 points on the dark adaptation curve, each point being the average of 5 measurements.

Fig. 4. Relation between temperature and the velocity constant \( k \) of the dark adaptation reaction. The straight line represents the Arrhenius equation.

The effect of temperature on dark adaptation in \( Mva \), therefore, not only shows the process to be bimolecular at all temperatures investigated, but demonstrates as well that the velocity constant of the process bears a well known theoretical relation to the temperature such as is usually found in chemical reactions.
SELIG HECHT

IV.

Ciona intestinalis.

1. Method.—In presenting the data obtained with Pholas and Mya only the barest manipulative essentials have been mentioned. Both species, though very sensitive to light, are hardy laboratory animals and tolerate much handling without change of sensibility. Therefore the methods used are simple and can easily be acquired anew by any one wishing to repeat the experiments.

The problem with Ciona, however, is more difficult. The species is not very sensitive to light, and in order to measure its dark adaptation a high illumination is required. This involves a still higher illumination for the initial light adaptation. The animals must therefore be brought close to a powerful source of light, such as a 1000 watt, gas-filled lamp of 2000 candle power. To maintain the animal at a constant temperature the usual heat screens are not enough, and a continuous addition of ice-cold sea water has to be maintained. On the other hand, Ciona is very sensitive to mechanical disturbances, and is easily stimulated by the necessary stirring of the water in the dish. Moreover, a sudden exposure to high illumination for light adaptation makes it contract vigorously; and in contrast to Pholas and Mya it does not relax immediately on continued illumination, and may remain contracted as long as 15 or 20 minutes. During this period its own water current is so small that stirring must be more continuous than usual, which serves to stimulate it mechanically and prolong the contracted state. Therefore to secure reproducible and significant data with Ciona it has been necessary to devise an especially careful mode of experimentation.

About 25 animals are isolated, each in a separate rectangular dish 5 cm. wide, 10 cm. long, and 7 cm. high, and kept in the dark overnight. Next morning their reaction time to a standard illumination is measured; as a result of which it is possible to select 5 or 6 animals which give a vigorous and clean-cut response to illumination, and which do so in about the same reaction time. Each animal so selected is then carried through the following steps.

It is light adapted. The intensity of the lamp is reduced by means of a rheostat until the filament barely glows. Ciona may be brought
as close as 30 cm. to such a light without responding. During a period of half an hour the current is increased up to 200 volts so gradually that the animal does not at any time contract to light, and at the end of the period it is illuminated with 10,000 meter candles. The temperature is kept constant by the very careful addition of small amounts of ice-cold sea water which is distributed by the vigorous current of the fully expanded animal. The animal remains at this high illumination for 6 minutes. At a given moment the illumination is discontinued sharply by means of a shutter, and dark adaptation begins. The manipulations from now on are carried on by the light of a dim ruby lamp to which *Ciona* is not sensitive.

The animal in its dish is placed in complete darkness in a thermostat and kept at a constant temperature for the next 7 or 8 hours. At certain times during dark adaptation the dish is carefully taken out and placed near the lamp in such a position that the animal will receive on exposure an illumination of 6000 meter candles. A minute is allowed to elapse; the animal is exposed by means of a shutter; its reaction time is recorded with a stop-watch; the light is turned off immediately; and the animal is returned to the thermostat. The exact moments when these measurements are made vary somewhat in the different series, but are the same for all the animals in a series. The first measurement is usually made 15 or 20 minutes after the beginning of dark adaptation; the remaining ones at hourly intervals thereafter. If it is desired to have readings closer together the entire procedure of light and dark adaptation is repeated the next day, and readings are made at intervals between those taken the day before.

2. Data.—After many preliminary experiments involving the elimination of errors and development of technic, I measured the dark adaptation of 61 individuals grouped in ten series of experiments. To illustrate the type of result secured with *Ciona* the detailed data of one series of experiments are given in Fig. 5. It is to be noted about Fig. 5 that (a) the points given are individual measurements of the reaction time, not averages; (b) each animal was measured on 2 successive days, three readings being made each day; (c) the curve drawn through the points for each animal is a theoretical one derived as will presently be described. This series is typical; five or six similar ones could be given.
FIG. 5. Dark adaptation of *Ciona*. Each point is a single reading of the reaction time of a single animal. With each animal the points were secured in two runs of dark adaptation; the first, third, and fifth were made on 1 day, the remaining three on the following day. The curve in each case is calculated from the equation of a bimolecular reaction.
The dark adaptation of *Ciona* is a very slow process (cf. Hecht, 1918–19 a), and takes about 7 or 8 hours for completion. Most of the measurements were not carried on for that length of time because of the almost unbearable tediousness of the observations. Three series (VIII, IXb, and XV) were, however, carried on to 6, 7½, and 6 hours, respectively. The data secured are given in Tables V, VI, and VII, and in Fig. 6.

3. Interpretation.—The mathematical treatment of the data of

![Graph](image-url)
Ciona is the same as of Pholas and of Mya, with the slight exception that the upper limit of the total velocity, instead of being taken as the latent period velocity, is taken as the maximum reaction time velocity.

**TABLE V.**

*Dark Adaptation of Ciona. Series VIII. 5 Animals. Temperature, 15.8°C. Reaction Time at Complete Dark Adaptation Is 2.10 Seconds.*

$k = 0.0336; a = 0.385.$

<table>
<thead>
<tr>
<th>Time in dark $t$ (min.)</th>
<th>Reaction time $r$ (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>6.54</td>
</tr>
<tr>
<td>60</td>
<td>3.74</td>
</tr>
<tr>
<td>120</td>
<td>3.14</td>
</tr>
<tr>
<td>180</td>
<td>2.84</td>
</tr>
<tr>
<td>240</td>
<td>2.66</td>
</tr>
<tr>
<td>300</td>
<td>2.52</td>
</tr>
<tr>
<td>360</td>
<td>2.39</td>
</tr>
</tbody>
</table>

The data as with Pholas and Mya fall on the familiar hyperbola obtainable at complete dark adaptation. This, like the latent period, is also an experimentally determined value.
corresponding to a bimolecular reaction. The curves in Fig. 6 are all calculated in this way, and they show, as do the comparisons in Tables V, VI, and VII, that the agreement between calculation and experiment is very good.

**TABLE VII.**

*Dark Adaptation of Ciona. Series XV. 6 Animals. Temperature, 16.5°C. Reaction Time at Complete Dark Adaptation Is 1.82 Seconds.)*

<table>
<thead>
<tr>
<th>Time in dark</th>
<th>Observed</th>
<th>Reaction time $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>sec.</td>
<td>sec.</td>
</tr>
<tr>
<td>22</td>
<td>4.77</td>
<td>4.77</td>
</tr>
<tr>
<td>60</td>
<td>3.35</td>
<td>3.36</td>
</tr>
<tr>
<td>120</td>
<td>2.67</td>
<td>2.70</td>
</tr>
<tr>
<td>180</td>
<td>2.53</td>
<td>2.44</td>
</tr>
<tr>
<td>240</td>
<td>2.31</td>
<td>2.29</td>
</tr>
<tr>
<td>300</td>
<td>2.24</td>
<td>2.21</td>
</tr>
<tr>
<td>360</td>
<td>2.13</td>
<td>2.14</td>
</tr>
</tbody>
</table>

**TABLE VIII.**

*Dark Adaptation of Frog Tadpole. Data from Obreshkove.*

4 Animals. Latent Period, 1.0 Second. $k = 0.00205$.

<table>
<thead>
<tr>
<th>Time in dark</th>
<th>Observed</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>sec.</td>
<td>sec.</td>
</tr>
<tr>
<td>10</td>
<td>52.3</td>
<td>52.3</td>
</tr>
<tr>
<td>20</td>
<td>30.0</td>
<td>28.7</td>
</tr>
<tr>
<td>30</td>
<td>20.6</td>
<td>20.6</td>
</tr>
<tr>
<td>40</td>
<td>15.2</td>
<td>15.3</td>
</tr>
<tr>
<td>50</td>
<td>12.8</td>
<td>12.8</td>
</tr>
<tr>
<td>60</td>
<td>12.7</td>
<td>10.8</td>
</tr>
</tbody>
</table>

*Obreshkove (1921), p. 268.

v.

**Tadpoles.**

1. *Data.*—In the course of some experiments on the photic responses of frog tadpoles, Obreshkove (1921) measured the dark adaptation
of 4 animals. The results are so regular that they must surely be of theoretical interest. Obreshkove, however, merely presents the data without entering into a study of their meaning.

The tadpoles, when exposed to illumination, execute a specific reaction in the nature of a sudden forward movement after a definite reaction time. This response, curiously, is not mediated through the eyes, because removal of both eyes does not alter the sensibility of the animal either qualitatively or quantitatively.

The method of measuring dark adaptation employed by Obreshkove is identical with that described for Ciona, Mya, and Pholas. The animal is exposed to an illumination of 10 meter candles for 1 hour, after which it is placed in the dark and at regular intervals its reaction time to the same light of 10 meter candles is measured. The data are given in Table VIII and Fig. 7, in which each point is the average of four readings, one with each of 4 animals.
2. Interpretation.—The theoretical treatment of these data is identical with those of Pholas and Mya. Dark adaptation conforms to the kinetics of a bimolecular process. The agreement between experimental and calculated values is shown both in Table VIII and in Fig. 7, where the smooth curve is the course of the process computed in the same manner as with the other animals.

The actual handling of the data involves the difficulty that the latent period has not been measured accurately. Obreshkove recognized the existence of a latent period, and tried to determine its magnitude by measuring the reaction time to comparatively high intensities, when the reaction time is almost entirely latent period. For the reaction time to the maximum intensity (500 meter candles) he records an average of 0.76 second, which he treats as the upper limit for the latent period.

This datum must be considered doubtful for two reasons. In the first place the measurements were made with a stop-watch. This instrument is obviously incapable of recording accurately values below 1 second in the hands of any experimenter, because of the time lost from eye to hand, and from the beginning of the finger contraction to the releasing of the watch movement. The eye-to-hand time is about 0.2 second. If the starting lag of the stop-watch is added to this, it makes a period which cannot be neglected in stop-watch records of less than 1 second, and renders the value of such records very uncertain. In the second place, the average value of 0.76 second for the reaction time is apparently derived by the dubious process of not counting the values which are above 1.0 second. Obreshkove gives the 29 individual measurements for this particular intensity. Of them 22 are below 1.0 second; 7 are above. By omitting these 7 one gets 0.76 as an average. The average of all the experimental values, since there seems to be no reason for omitting any, is 0.94 second. Neither of these values is an accurate measure of the latent period. But they indicate that its duration is of the order of 1 second.

Fortunately this is all that is necessary. The values of the reaction time during dark adaptation are quite large: the longest is 52.3 seconds, and the shortest is 12.7 seconds. An error of even 0.5 second in the latent period introduces an error of 1 per cent and 4 per cent,
respectively, in the maximum and minimum exposures. I have therefore assumed the latent period to be 1.0 second and have used this value in computing the figures in Table VIII and the curve in Fig. 7. Actually, if the latent period be assumed as 0.7 second or as 1.3 second it makes no noticeable difference in the computed values as given in this table and figure.

The data of the dark adaptation of the tadpole are therefore represented by the isotherm of a bimolecular reaction, and show the adequacy of the theoretical treatment to which they have been subjected.

VI.

CONCLUSION.

1. Reaction Order.—At first sight it may seem strange that the 3 animals here investigated and the 1 studied by Obreshkove should all show a dark adaptation whose kinetics correspond to the same order of reaction. This is not surprising, however, on intimate acquaintance with the animals, because the similarity of their photosensory make-up is apparent in a variety of ways: in their response to light; in the composition of their reaction time; in the effect of temperature on the two parts of their reaction time; etc. The identity of the order of the reaction underlying dark adaptation is thus only one more property which points to a basic similarity of the photosensory process in these species.

This must not be interpreted to mean that the actual substances entering into the reaction are identical in all the species. In fact, there is evidence—such as the specific spectral sensitivity, and the specific effects of temperature, to be published shortly—which shows that these substances are not the same. It is merely their arrangement and the order of their reaction—in short their organization—which seem to be identical in the different animals.

That the reaction underlying dark adaptation is bimolecular and not monomolecular is not unexpected. Dark adaptation is a process in the course of which a sensitive material accumulates in the sense cell as the result of a chemical reaction. If one supposes that the sensitive material is built up mainly from the products of its photolysis, then a bimolecular reaction is almost the simplest process conceivable.
2. Photosensory Mechanism.—The idea of such a reversible system has been at the basis of all the work which has grown out of the original experiments with *Mya*, and which in turn have served to define that system more rigorously.

It is supposed that a photosensitive material, *S*, is decomposed by light into at least two substances, *P* and *A*, which at the same time tend to recombine and form the original material, *S*. This reversible reaction has been referred to as the primary photochemical reaction. By itself it is sufficient to account for only a part of the characteristics of the photosensory process in these animals. The existence of a latent period whose duration is dependent on the primary photochemical reaction calls for an additional process in the sense cell. An inactive substance, *L*, is converted into an active form, *T*, a definite accumulation of which sets off, electrically or chemically, the nerve-ending attached to the sense cell and thus starts the train of events culminating in the specific response of the organism. This latent period reaction *L* → *T* can proceed only in the presence of *P* and *A* freshly formed from *S* by the primary photochemical reaction. This relation is probably a simple catalysis.

In a diagrammatic way these two interrelated reactions may be written

\[
\text{light} \quad S \xrightleftharpoons{\text{light}} P + A; \quad L \parallel P + A \parallel \rightarrow T \quad \text{impulse}
\]

where \( P + A \parallel \) means catalysis by *P* + *A*. The rectangle represents the sense cell. Light comes in at one end and the impulse for the nerve leaves the other end. The two reactions are given here as separated in space; it is to be understood that they are intimately mixed in the cell.\(^5\)

\(^5\) The following statement occurs in a recent paper by Folger (1926, p. 368).

"Mast ('07) was among the first to postulate a reversible photochemical process to account for photic response in lower organisms. Hecht ('19) elaborated this idea to explain the response to light in *Mya arenaria*." In order to make clear the developmental sequence of the ideas here involved, the complete statement of Mast's hypothesis, taken from the paper referred to (Mast, 1907, p. 159), is given verbatim. "To explain reversal in the sense of reaction on the basis of chemical reactions induced by light let us assume: (1) That Volvox contains substances X and Y,...."
The primary reaction, here written as a completely reversible reaction

\[
\text{light} \quad S \rightleftharpoons P + A \quad \text{"dark"}
\]

may indeed be only a pseudoreversible reaction

\[
\text{light} \quad S \rightarrow P + A + B \quad \text{"dark"}
\]

\[
S \leftarrow P + A + C
\]

in which \( B \) is the substance that actually controls the reaction \( L \rightarrow T \), and \( C \) is a substance present in excess. In picturing concretely the behavior of such a system as given in the above rectangular diagram the pseudoreversible reaction is often more helpful than the simpler, completely reversible one. Mathematically, the two are identical.

Such a coupled system of two reactions describes most of the features exhibited by the photosensory process, not merely qualitatively but in quantitative detail as well. It accounts for the composition of the reaction time; for the different effects of temperature on the two parts of the reaction time; and for the interrelation of the two parts of the reaction time. It accounts for the spectral sensitivity of the animals; for the kinetics of dark adaptation, as we have seen; and for the effect of temperature on dark adaptation. It accounts for light adaptation and sensory equilibrium; for the sensitivity changes at different light levels; and for intensity discrimination. It even includes the proof of the major assumption made in the theoretical treatment of dark adaptation, namely that under all conditions of adaptation a constant photochemical effect is necessary.

Chemical reaction between which is regulated by the intensity of light: (2) that a sub-optimum intensity favors the formation of substances represented by \( X \) and a supra-optimum intensity those represented by \( Y \); and (3) that the colonies are neutral in reaction when there are \( Y \) substances in one member of the equation and \( X \) in the other; positive when one member contains \((X+)\) substances and the other \((Y-)\), and negative when one contains \((X-)\) and the other \((Y+)\).” Probably the first use of the idea of a reversible photochemical reaction in relation to the photosensory process was made by Müller (1896).
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to produce a definite response in the animal. The general idea involved therefore leads to a consistent and rational view of the nature of the photosensory process.

VII.

SUMMARY.

1. Data are presented for the dark adaptation of four species of animals. They show that during dark adaptation the reaction time of an animal to light of constant intensity decreases at first rapidly, then slowly, until it reaches a constant minimum.

2. On the assumption that at all stages of adaptation a given response to light involves a constant photochemical effect, it is possible to describe the progress of dark adaptation by the equation of a bimolecular reaction. This supposes, therefore, that dark adaptation represents the accumulation within the sense cells of a photosensitive material formed by the chemical combination of two other substances.

3. The chemical nature of the process is further borne out by the fact that the speed of dark adaptation is affected by the temperature. The velocity constant of the bimolecular process describing dark adaptation bears in My a relation to the temperature such that the Arrhenius equation expresses it with considerable exactness when \( \mu = 17,400 \).

4. A chemical mechanism is suggested which can account not only for the data of dark adaptation here presented, but for many other properties of the photosensory process which have already been investigated in these animals. This assumes the existence of a coupled photochemical reaction of which the secondary, “dark” reaction is catalyzed by the products of the primary photochemical reaction proper. This primary photochemical reaction itself is reversible in that its main products combine to form again the photosensitive material, whose concentration controls the behavior of the system during dark adaptation.

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