PHOTOCHEMISTRY OF VISUAL PURPLE.

III. THE RELATION BETWEEN THE INTENSITY OF LIGHT AND THE RATE OF BLEACHING OF VISUAL PURPLE.

BY SELIG HECHT.*

(From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston.)

(Received for publication, May 23, 1924.)

I.

1. In the reception of light by the eye a common variable is the intensity of the illumination. Indeed, in order to analyse phenomena like dark adaptation and intensity discrimination it has been necessary to assume (Hecht, 1921–22, c; 1923–24, d) certain relations between the intensity of light and its action on the photosensitive material in the eye.

The idea that visual purple is the sensitive substance concerned with rod vision has had support in an interesting array of quantitative evidence (Koenig, 1894; Trendelenburg, 1904; Hecht and Williams, 1922–23). More recently (Weigert, 1921, a; 1922, b; Hecht and Williams, 1922–23) it has even seemed possible that visual purple may be concerned with cone vision as well. It is therefore pertinent to a study of the visual process to determine experimentally the influence of the light intensity on the bleaching of visual purple.

2. The chemical change produced by light is proportional to the amount of energy absorbed by the sensitive material (von Grotthuss, 1819; Lasareff, 1907; Luther and Weigert, 1905; Plotnikow, 1907; Weigert, 1922, c). The form which this relation takes with visual purple is a simple one. Let $a$ be the initial concentration of visual purple, and let it be exposed for the time $t$ to light of intensity $I_0$ in

* National Research Fellow.
such a way that it absorbs an amount \( I_a \), and as a result the quantity \( x \) is bleached. Then (Plotnikow, 1920)

\[
\frac{d(a - x)}{dt} = k I_a
\]  

where \( k \) is a constant. The relation between the incident light \( I_0 \) and the absorbed light \( I_a \) is according to Beer's law

\[
I_a = I_0 (1 - e^{-m(a-x)})
\]  

\( m \) being the absorption coefficient. Since the concentration of visual purple in the solution used in these experiments is quite small (cf. Garten, 1907) equation (2) may be simplified. The exponential \( e^{-m(a-x)} \) may be expanded into the series

\[
\sum_{n=0}^{\infty} \frac{(-1)^n m^n (a-x)^n}{n!}
\]  

Since \( (a-x) \) is small, it is safe to neglect all the terms in equation (3) which involve powers higher than unity. The series then reduces to \( 1 - m(a-x) \) and equation (2) becomes

\[
I_a = I_0 m (a-x).
\]  

This value for \( I_a \) may now be substituted in equation (1). Remembering that the incident intensity \( I_0 \) remains constant in a given experiment, we may write

\[
K = k m I_0.
\]  

Equation (1) then becomes

\[
\frac{d(a-x)}{dt} = K(a-x)
\]  

which when integrated gives

\[
K = \frac{1}{t} \log \frac{a}{a-x}
\]  

the familiar exponential law corresponding to the kinetics of a reaction of the first order.

3. These deductions involve two steps which can be verified ex-

\(^1\) Garten (1907), p. 168.
One is that the bleaching reaction follows a monomolecular course (equation (7)). In the first paper of this series, and indirectly in the second as well (Hecht, 1920–21, a, b) evidence was presented which shows that this is true within the limits of error of the colorimetric method. This, as is clear from the above equations, means that the rate of bleaching at any moment is proportional to the amount of light absorbed, which in turn is a function of the concentration (equation (4)).

Another step in the above reasoning is the assumption that the velocity constant $K$ of the bleaching reaction is directly proportional to the intensity of illumination (equation (5)). It is the purpose of the present work to test this experimentally.

II.

1. The experiments were done by two different methods. Three groups of experiments were made with a colorimetric method and a fourth group with a spectrophotometric method. The colorimetric method has already been described in the first paper of this series. It consists essentially in estimating the concentration of visual purple exposed to light by matching its color in a capillary tube against standards made up of a series of relative concentrations of bleached and unbleached visual purple contained in similar capillary tubes. A set of experiments, made in a single day, consists of measurements of the course of bleaching of visual purple at a number of different intensities. Group I consists of four such sets of experiments; Group II of one set, and Group III of three sets.

2. Group IV, composed of five sets of experiments, was differently performed. A cell was constructed which allows spectrophotometric readings to be made with 1 cc. of solution. The cell serves as well for the vessel in which visual purple is exposed to light, so that no transfer of material is necessary. Exactly 1 cc. of visual purple solution is pipetted into the cell and its absorption measured at 520 $m\mu$, ten settings of the nicols being made for the purpose. The material is then exposed to a given intensity for a chosen time, during which the contents of the cell are stirred evenly and automatically by a motor-driven spirally twisted stirring rod. The cell is then removed and measured, after which it is replaced in front
of the bleaching light, exposed for a further length of time, and again measured. By means of preliminary experiments the exposures were adjusted to give the same amount of bleaching for the various intensities.

The absorption readings are made in terms of photometric density, $D$, which may be defined in terms of Beer's law (equation (2)) as

$$D = m(a - x) = \log \frac{I_s}{I_1}$$

where $I_s$ is the transmitted light. From this it is apparent that the concentration of a substance varies directly as its photometric density. The readings give the relative concentrations of visual purple present after specific exposures to light, provided a correction is made for the photometric density of the material remaining after the visual purple has been completely bleached. A set of experiments is made in 1 day with the same preparation of visual purple.

The absorption measurements are made at 520 $m\mu$ with a Hilger constant deviation spectrophotometer of the Nutting type. By placing a green filter (Wratten No. 63) between the source of light and the cell, only the part of the spectrum around 520 $m\mu$ falls on the visual purple solution. The wave length 520 $m\mu$ was chosen for measurement because it lies between the maximum absorption of visual purple and the maximum visibility of the spectrum for the fovea and because the absorption represents mostly visual purple and comparatively little of the additional yellow materials which are extracted with it from the retina by the bile salts or are formed from it as an intermediary product of bleaching (Kühne, 1879, Koenig, 1894; Köttgen and Abelsdorff, 1896; Garten, 1907). The source of light for the measurements is a 15 watt concentrated filament lamp, which is kept on only during the actual setting of the nicols.

All the work, and all the readings are made in a dark room, and the spectrophotometer is so arranged that the cell is shielded from every possible stray illumination. As a result of these precautions it is possible to make a large number of readings without perceptibly changing the absorption of the solution. In an experiment made to test this point, forty successive settings of the nicols were recorded.
in the usual way in groups of ten. The four averages of the photometric density are 0.376, 0.375, 0.375, and 0.379, showing that the light used in measurement is not a source of error.

3. The bleaching intensities, ranging between 10 and 1,000 meter candles are obtained by varying the distance of the visual purple from a concentrated filament lamp. In Groups I and II the source was a 250 watt lamp; in Group III it was a 500 watt lamp; and in Group IV it was a 400 watt lamp. The intensities were calculated by the inverse square law.

4. The experiments were made at four different times, each group representing a separate period of experimentation. The solutions of visual purple were prepared so that the experiments within the individual groups involve similar concentrations. The absolute values of the concentrations are unknown at present because visual purple has never been isolated as a pure substance. The concentrations in Groups I and II represent four frog retinas to 1 cc. of solution; in Group III about three retinas to 1 cc. of solution; and in Group IV about one retina to 1 cc. of solution. The colorimetric method uses about 0.1 cc. for each experiment, whereas the spectrophotometric method requires about 1 cc., but of a diluter solution.

III.

1. The results are presented in Table I. Each figure in Group I is the average value of $K$ for four experiments; in Group II for one experiment; in Group III for three experiments; and in Group IV for five experiments. It is apparent from the table that the velocity constant of the bleaching reaction varies directly with the intensity of the illumination.

To show the exact nature of this relation the data are plotted in Fig. 1, from which it is clear that the velocity constant is a linear function of the intensity. This is true not only for the experiments as a whole, but for the individual groups as well. The separate groups are identifiable in Fig. 1, and examination of their respective points will show that each group is best represented by a straight line.

2. A word of explanation of the mode of presentation of the data is necessary. The values in Table I of the velocity constants of
Group I are given as computed from the original observations by means of equation (7). Since, however, the amount of solution used as well as the surface exposed to the light varies slightly from group to group, the velocity constants calculated from the different series of experiments are not identical, though nearly so. In order to present them here, the velocity constants of the other three groups are multiplied by a factor, as given in Table I for each group. The factors are arbitrarily chosen as follows. The data from each group are plotted and a straight line drawn through them. The factor then is the fraction by which the tangent of the line must be multiplied in order that the line will coincide with that drawn for Group I.

This procedure does not in the least influence the nature of the results. The numerical values of the velocity constants have no theoretical meaning by themselves in the present state of our knowledge of visual purple; it is only their relative values which are of significance. And the present experiments leave little doubt that

\[
K = \frac{1}{\alpha} \log \frac{a}{\alpha - s}
\]

are plotted and a straight line drawn through them. The factor then is the fraction by which the tangent of the line must be multiplied in order that the line will coincide with that drawn for Group I.

This procedure does not in the least influence the nature of the results. The numerical values of the velocity constants have no theoretical meaning by themselves in the present state of our knowledge of visual purple; it is only their relative values which are of significance. And the present experiments leave little doubt that

<table>
<thead>
<tr>
<th>Intensity</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>m. c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>0.00774</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.0</td>
<td></td>
<td>0.0148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.5</td>
<td>0.0144</td>
<td>0.0148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52.5</td>
<td>0.0277</td>
<td>0.0267</td>
<td>0.00454</td>
<td></td>
</tr>
<tr>
<td>60.0</td>
<td></td>
<td></td>
<td></td>
<td>0.0281</td>
</tr>
<tr>
<td>100.0</td>
<td></td>
<td></td>
<td>0.0395</td>
<td>0.0469</td>
</tr>
<tr>
<td>110.0</td>
<td>0.0463</td>
<td>0.0566</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200.</td>
<td></td>
<td></td>
<td>0.0732</td>
<td></td>
</tr>
<tr>
<td>250.</td>
<td>0.120</td>
<td>0.102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300.</td>
<td></td>
<td></td>
<td>0.136</td>
<td>0.127</td>
</tr>
<tr>
<td>500.</td>
<td></td>
<td></td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td>510.</td>
<td>0.229</td>
<td>0.237</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600.</td>
<td></td>
<td></td>
<td></td>
<td>0.258</td>
</tr>
<tr>
<td>690.</td>
<td></td>
<td></td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>1,000.</td>
<td>0.439</td>
<td></td>
<td>0.447</td>
<td>0.452</td>
</tr>
</tbody>
</table>
the velocity of bleaching of visual purple under comparable conditions of concentration, volume, and surface exposed is directly proportional to the intensity of the incident light.

![Graph showing the relation between the velocity constant $K$ of the bleaching reaction and the intensity of the light.](image)

Fig. 1. Relation between the velocity constant $K$ of the bleaching reaction and the intensity of the light.

IV.

1. The results of these experiments are a confirmation of the Bunsen-Roscoe law. The validity of this law has been questioned by those who have tested it on complicated reactions like the photographic process (Schwarzschild, 1899). However, as originally con-
ceived by Bunsen and Roscoe (1862) it probably applies only to simple irreversible photochemical reactions. The bleaching of visual purple under the conditions of these experiments is an example of such a process. Equation (7) may, as a result of the present experiments, be written

\[ k \, m \, I = \frac{1}{t} \log \frac{a}{a-x} \]  

which can then be changed to

\[ I \, t = \frac{1}{k \, m} \log \frac{a}{a-x} \]  

The Bunsen-Roscoe reciprocity law requires that in order to produce a definite photochemical change in a given system the product of the intensity \( I \) and the time of its action \( t \) be constant. For a constant amount of bleaching \( \log \frac{a}{a-x} \) is constant. And since \( k \) and \( m \) are both constants, the right hand member of equation (10) becomes constant and the equation reduces to the usual form of the Bunsen-Roscoe law

\[ I \, t = \text{constant.} \]  

2. In the present experiments the bleaching of visual purple is an irreversible process. However, in the eye, the bleaching of this pigment is distinctly not an irreversible process. Even in the isolated retina, and occasionally in solution, Kühne showed years ago (1879) that visual purple is regenerated in the dark. The system is therefore a reversible one. Very likely, as Luther and Plotnikow (1908) suggested, it is a pseudoreversible reaction in which one of the substances necessary for regeneration has to be freshly supplied. However, since with an excess present of the accessory substance necessary for the dark reaction, the behavior of the pseudoreversible reaction simulates a completely reversible reaction, it is advisable to be non-committal and call the visual purple system in the retina simply a reversible one, the chemical conditions for regeneration being at present unknown.
The effect of light on such a reversible system is less than on the same system in an irreversible state, and the difference depends on the speed of the dark reaction. Certain evidence (Hecht, 1921–22, c) leads us to suppose that the kinetics of the "dark" reaction are bimolecular, perhaps trimolecular. The quantitative results of such a situation have already been gone into elsewhere (Hecht, 1923–24, d) and need not be repeated here. They indicate that some properties of vision can be described quantitatively in terms of a reversible reaction of this nature. One assumption in this system has been that the velocity constant of the bleaching reaction _per se_ is a linear function of the intensity. This is now supported by the present experiments.

**SUMMARY.**

It is shown that the velocity of bleaching of visual purple by light, under comparable conditions of concentration, volume, and surface exposed, is directly proportional to the intensity.

**BIBLIOGRAPHY.**


PHOTOCHEMISTRY OF VISUAL PURPLE. III


Trendelenburg, W., Quantitative Untersuchungen über die Bleichung des Sehpurpurs in monochromatischem Licht, Z. Psychol. u. Physiol. Sinnesorg., 1904, xxxvii, 1.