DARK ADAPTATION IN AGRIOLIMAX.

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1.

When the slug Agriolimax, previously dark adapted, is allowed to creep geotropically upward upon a vertical plate, lateral illumination forces it to pursue a path at an angle $\beta$ to the perpendicular (cf. Wolf, 1926-27) and away from the light. The magnitude of this angle decreases with time (Wolf and Crozier, 1927-28), due to photic adaptation in the eye (Crozier and Wolf, 1927-28). The change with time during continuous exposure is adequately accounted for, quantitatively and very simply, by the assumption that the path at any moment is the resultant of 2 vectors, respectively those determined by geotropic and by phototropic excitation, and that these vectors express quantitatively the effects of the corresponding excitations (Wolf and Crozier, 1927-28). The geotropic excitation is taken to be constant, while the phototropic excitation decreases exponentially with time, due to the first order character of the light adaptation. These assumptions lead to an expression for $\beta$ as a function of time,

$$\text{time} = a - \frac{1}{K} \log \tan \beta,$$

which is very satisfactorily obeyed; and to the expectation that the rate of adaptation, $K$ in equation (1), should be practically a logarithmic function of the light intensity (over intermediate ranges), which is also substantiated. The assumptions underlying this treatment are that the intensity of excitation is proportional to $-\frac{ds}{dt}$ where $S$ is the substance which is decomposed photochemically,

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and that with the animals fully dark adapted at the start \( \frac{ds}{dt} \) is effectively proportional to \([S]\) at each moment, any reverse reaction serving to regenerate \( S \) being negligible under the conditions (at lower temperatures, when dark adaptation is known to be quite slow). It was pointed out (Wolf and Crozier, 1927–28) that this type of experiment can be reversed for the study of dark adaptation. It is important to determine the characteristics of the receptor mechanisms concerned with continuous excitation by light, as in phototropism, and we have accordingly made experiments regarding the kinetics of dark adaptation in \textit{Agriolimax}. The continuous nature of the photic excitation is proved by the character of the path of orientation. The orientation is fully accounted for by the assumption that the difference in tonus of the musculature which results in turning is proportional to the photic excitation. The results demonstrate one further respect in which the receptor machinery for phototropic excitation and that for differential sensitivity, as studied by Hecht (1918–19; 1926–27) in several forms, are fundamentally akin. They may also be invoked as supplying an additional chapter in the development of the prediction of conduct in compound fields of stimulation (Crozier, 1923–24; 1926–27; Crozier and Pincus, 1926–27; Crozier and Stier, 1927–28).

\textit{Agriolimax} was allowed to become light-adapted by exposure for at least 2 hours to bright daylight at a window facing an unclouded sky. It is necessary to make sure at intervals that the slugs are creeping, with eye-stalks everted. Exposure to direct sunlight is quickly injurious, but illuminations of about 1,000 m. c. proved non-toxic and adequate \( (\text{i.e., sufficiently high}) \) for our purpose. Light-adapted individuals were then taken in succession and placed in darkness for 5 minutes each. A curve of light adaptation was then obtained for each, by measuring the divergence from perpendicular creeping upon a moist vertical plate of ground glass, as earlier described (Wolf and Crozier, 1927–28). The adapting light was of 29.4 f. c. The data from a sample of 10 individuals treated in this way show good agreement, and the readings of the angle of divergence \( (\phi) \) at successive minutes during light adaptation are accordingly averaged. The
TABLE I.
Amplitudes of response ($\beta$) at successive intervals in lateral light of 29.4 f.c. intensity, beginning at various levels of dark adaptation. (The magnitudes of $\beta$ have been checked by further groups of experiments.)

<table>
<thead>
<tr>
<th>Time in darkness (min.)</th>
<th>No. of individuals</th>
<th>Time in light (min.)</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>31.98°±0.995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>21.23°±1.00</td>
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<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>13.07°±1.02</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>0.5</td>
<td>40.28°±1.208</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>26.34°±0.605</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>18.97°±0.477</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>12.62°±0.639</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>0.5</td>
<td>44.06°±1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>51.87°±0.702</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>25.0°±0.838</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>18.0°±0.526</td>
</tr>
<tr>
<td>15</td>
<td>17</td>
<td>0.5</td>
<td>59.45°±1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>45.80°±1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>34.30°±1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>25.80°±1.02</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0.5</td>
<td>58.0°±1.25</td>
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<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>46.63°±1.14</td>
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<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>35.81°±0.940</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>26.44°±1.05</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>0.5</td>
<td>59.25°±1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>42.60°±1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>30.25°±1.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>20.90°±1.24</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>0.5</td>
<td>61.60°±1.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>49.90°±1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>34.62°±0.723</td>
</tr>
<tr>
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<td></td>
<td>3.5</td>
<td>25.43°±0.821</td>
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<td>17</td>
<td>0.5</td>
<td>62.50°±1.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>47.2°±0.936</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>34.33°±1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>24.89°±0.529</td>
</tr>
<tr>
<td>60</td>
<td>14</td>
<td>0.5</td>
<td>62.50°±1.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>47.2°±0.936</td>
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<td></td>
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<td>3.5</td>
<td>24.89°±0.529</td>
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</table>
tests are then repeated with similar slugs each dark-adapted for 10 minutes, and so on for succeeding intervals up to 60 minutes (Table I). The temperature in the dark room was held at 20.5° to 22.0° throughout. At this temperature the rate of increasing effectiveness of photic excitation is still very slow by comparison with that of adaptation by light.

It is impossible to carry through such series of observations, or rather to interpret the results in a simple and direct way, when single individuals are tested at different times during their dark adaptation (as subsequently discussed), and physical limitations make it necessary to deal with relatively small groups of individuals at a time (their respective intervals in darkness being properly spaced and controlled). The data for each curve of light adaptation has therefore been based, as a rule, upon a group of ten slugs. Individual differences, of quantitatively perceptible order but of consistent sense, expose the interpretation of such data to certain hazards. At each desired point on the final curve of dark adaptation, therefore, the tests were repeated with new groups of individuals. Aside from possible but slight variations in the intensity of the test light, and of the adapting illumination, the existence of diurnal variations in photic excitability must be guarded against; and, more important in a practical way, diurnal fluctuations in geotropic responsiveness. It is known, from our own experience with Agriolimax and other slugs, that the geotropic response is more powerfully in evidence, or—what may not be the same thing—more frequently expressed,—toward evening and after nightfall than in the early morning hours. Diurnal fluctuations of activity have long been noted in other forms. In our earlier work such changes have been discounted by running the experiments between hours 11 a.m. and 6 p.m., and by using a large number of series of observations spread more or less uniformly over this interval.

The derivation of equation (1) assumed that $g$, the geotropic vector, remained constant. It is also assumed that the threshold value of the photic excitation, required to produce any phototropic orientation on the vertical plate, is not a value to be subtracted from the total photic excitation, but that if effective at all the photic stimulation has its full proportionate value. Questions concerning the central nervous interplay between photic and gravitational stimulations receive some
further elucidation in experiments subsequently made with Limax, to be treated in another place. Variations in this threshold quantity would not affect the intercept on the t axis in Fig. 1, nor the slope of the curve of light adaptation.

We have been fully alive to the fact that in treating the photic excitation no account has been taken of the inclination of the receptive surface to the path of the light. One can suspect that change in this angle would alter the magnitude of our L. But the question is complicated by the fact that the eye-tentacles of Agriolimax diverge further as light adaptation increases, so that the angle between the eye-surfaces ("H" of a previous paper—Crozier, 1925–28) decreases as the slug comes to orient more nearly straight upward. This would tend to increase L illegitimately, and to cause curves such as those in Fig. 1 to be slightly concave upward. In a number of instances this appears to be true, and in no instance is the reverse true. But the effect, if real, is of small magnitude, with the orienting light of moderate intensity; and it may possibly be accounted for in other ways (cf. § VII).

III.

The notion that excitation by light is a photochemical matter, and that the maintenance of photic reactivity and its recovery after exhaustion may be in some fashion connected with a reversible photochemical receptor system seems to have been held in a very general form since Hering. Growing knowledge of the chemical effects of light made it attractive to translate such ideas into photochemical terms. An early attempt to treat the question precisely and specifically, but with some important deficiencies, was made by Müller (1896). To explain the simultaneous exhibition of capacity to react to a sharp decrease in light intensity and also to be excited to phototropic movements by the continuous action of light, as seen in the integument of certain holothurians, it was suggested that the same photochemical system, if part of a reversible reaction of a certain kind, might be conceived to serve for both modes of stimulation (Crozier, 1915). Hecht has been able to achieve the step-by-step proof, in a very satisfying and convincing manner, that a particular

1 Hecht, 1918–19; 1919–20, a, b; 1922–23; 1925–28; 1926–27.
type of reversible photochemical system underlies photic excitation of *Mya, Ciona, Pholas,* and the human eye. The properties of this system permit the prediction and the rational quantitative interpretation of new and unexpected characteristics of the stimulation process. A major property of the underlying mechanism is adequately expressed (Hecht, 1918–19; 1922–23) in the following paradigm, where $S$ represents primary photosensitive substance, $P$ and $A$ two of the products of its photolysis:

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

The system is reversible, and with time, under continuous constant illumination, there is established a stationary state ("equilibrium"). The "dark" process, of which the kinetics serves to describe the course of dark adaptation, is definitely bimolecular (Hecht, 1926–27), with high temperature characteristic; the "light" reaction is of first order and negligibly influenced by temperature (Hecht, 1919–20, a).

These relationships have been established most elaborately by means of experiments with organisms in which the speed of response following the delivery of a measurable quantity of light gives data necessary for the quantitative treatment, and in which light adaptation is rapid and photic excitation therefore necessarily discontinuous. To carry over such considerations to the analysis of continuous excitation, as in phototropic stimulation, it has been necessary to find phototropic organisms in which the rate of light adaptation is measurable, neither inconveniently rapid nor too slow. The photic adaptation of *Agriolimax* has been followed by causing the phototropic influence of the light to work against the "brake" provided by a vectorially constant excitation of a different kind, namely that due to gravity, acting at right angles to the phototropic vector. The function of such a "brake" in contributing to the significance of the measured orientations has been discussed previously in relation to circus movements (Crozier and Federighi, 1924–25). Data necessary for treatment of the light adaptation are gotten from measurements of the resultant angles of orientation as related to time and to intensity (Wolf and Crozier, 1927–28). Such measurements are possible because the rate of light adaptation, especially at temperatures in the neighborhood of $15^\circ$ or slightly above, is so very much faster than
that of dark adaptation, as adequate tests showed. It was easily established that the rate of light adaptation is very little influenced, if at all perceptibly, by temperature; and that its course follows that of a first order reaction in which the "velocity constant" is a linear function of the logarithms of the light intensity. These phenomena therefore parallel in an exact manner the properties of the "light" reaction already discussed.

The interpretation of dark adaptation requires measurements of the photic excitability at successive intervals following exclusion of light from individuals previously light-adapted. To reduce the probability of adventitious errors of estimation the experiments were made at a temperature high enough to bring the time for practically complete dark adaptation down to a little over 1 hour. At about 12°, the time required may be as long as 3 to 4 hours; at 20.5°–22°, the temperature prevailing throughout the present experiments, the time is less than half of this. Since light adaptation, as measured by the power to influence geotropic creeping on a vertical surface, is effectively complete within about 5 minutes, as a rule, with high intensities, the experiments must, for simplicity, take the form of establishing curves of light adaptation as affected by known preceding periods in darkness. From such data it is required to deduce the ideal photic excitability at the last moment in darkness, before the exposure has begun, or in other words the kinetics of the "dark" reaction. The necessity for speedy and precise manipulation has made it undesirable, for the present, to vary the light intensity from moment to moment in such a way as to keep the angle $\beta$ constant. This alternative method has certain theoretical advantages, however, and subsequently will be used.

IV.

Two aspects of the measurements at once present themselves—the rates of light adaptation after various periods in the dark, and the magnitudes of $\beta$ at the first instant of exposure to the light. It is apparent from Fig. 1 that the course of light adaptation after given conditions of adjustment in the dark adheres to the equation previously obtained,

$$\text{time} = a - \frac{1}{K} \log_{10} \tan \beta,$$  (2)
Fig. 1. The angle (θ) of orientation of *Agriolimax* away from the vertical path on a perpendicular plate, as enforced by exposure to horizontal light of 29.4 f.c. on the right side, at successive minutes during the exposure, following increasing residence in darkness (5 to 60 minutes) of slugs initially light-adapted. Time of exposure plotted against log tan θ gives graphs sensibly rectilinear. The intercepts of these graphs on the ordinate axis give a means of estimating the photic excitability at the very first moment of exposure to light (see text). (The ordinate axis has been shifted vertically, for “30 min.” and for “45 min.” and “60 min.” to obviate overlapping.)
and that $K$, the rate coefficient of light adaptation, and $a$, the intercept on the $\beta$ axis corresponding to zero time of exposure to light, each vary with the extent of dark adaptation. From the assumptions made and tested in the previous papers, we may write

$$\tan \beta = \frac{L}{g}$$

(3)

where $L$ and $g$ represent respectively the photic and the gravitational orientation vectors. If $g$ is constant, $L = g \tan \beta$. $L$ is taken to be a measure of the average amount of photosensory substance present,

**TABLE II.**

Intercepts on the log$_{10}$ tan $\beta$ axis (i.e., log$_{10}$ tan $\beta_1$) at $t = 0$ exposure to light (29.4 f.c.), following increasing residence ($t$) in darkness of initially light-adapted slugs. $R = \frac{KL_\infty}{KL_t}$; $KL_\infty$ = intercept for completely dark-adapted animals.

<table>
<thead>
<tr>
<th>$t$ (min)</th>
<th>log$_{10}$ tan $\beta$</th>
<th>$\beta_1$ (°)</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9.910-10</td>
<td>39.1°</td>
<td>3.020</td>
</tr>
<tr>
<td>10</td>
<td>9.996</td>
<td>44.7°</td>
<td>2.477</td>
</tr>
<tr>
<td>15</td>
<td>10.059</td>
<td>48.9°</td>
<td>2.143</td>
</tr>
<tr>
<td>20</td>
<td>10.216</td>
<td>58.7°</td>
<td>1.528</td>
</tr>
<tr>
<td>25</td>
<td>10.264</td>
<td>61.5°</td>
<td>1.337</td>
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<td>10.305</td>
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<td>45</td>
<td>10.350</td>
<td>65.9°</td>
<td>1.096</td>
</tr>
<tr>
<td>60</td>
<td>10.362</td>
<td>66.5°</td>
<td>1.067</td>
</tr>
<tr>
<td>$\infty$</td>
<td>10.390</td>
<td>67.9°</td>
<td>1.000</td>
</tr>
</tbody>
</table>

$s$, or more precisely as the average value of $-\frac{ds}{dt}$, during the interval over which $\beta$ is estimated. This is the amount of substance the increase of which, in terms of the conception of a reversible photo-chemical system, we desire to follow during dark adaptation. The two assumptions as to the nature of $L$ will be tested separately.

Taking the intercepts on the log$_{10}$ tan $\beta$ axis of Fig. 1, and from these obtaining $\tan \beta$, we have numbers proportional to the concentrations of $S$ present at the beginning of the test-exposures, if the "dark" reaction is relatively negligible; this follows from the primary assump-
tion (Wolf and Crozier, 1927-28) that the phototropic excitation in any short interval is proportional to $-\frac{ds}{dt}$ and thus to the amount of $S$ photolysed in unit time. Strictly speaking, this of course may not be entirely accurate, since the angle $\beta$ is estimated over a finite length of time and of path; but deficiencies arising in this way are lost among the individual variations of response and are of no moment for our present purpose. The values of $\tan \beta$ corresponding to $t = 0$ increase regularly with time of residence in darkness (Table II and Fig. 2), and the adaptation is therefore an orderly process. The type of reaction which describes the kinetics of dark adaptation, and thus of the "dark" reaction in the absence of light, may be ascertained from the data in Table I and Fig. 2. Since $\tan \beta$ is proportional to $L$, the intensity of excitation, and this by assumption to $-\frac{ds}{dt}$ and

![Graph showing dark adaptation](image-url)
so to $S_t$, the amount of photosensitive substance present at the moment, we might write

$$\frac{\tan \beta_t}{\tan \beta_i} = \frac{L_t}{L_i} = R,$$

where $\beta_i$ is the angle given by the intercept on the log tan $\beta$ axis in Fig. 1, —i.e., corresponding to zero time in the light, after $t$ minutes dark adaptation, and $\beta_t$ is the intercept angle after complete dark adaptation. Thus we have a measure of the photic sensitivity at the first moment of exposure to the test light. From (4), since $L_t \propto S_t$, $S_t$ being the concentration of $S$ after time $t$ in the dark,

$$\frac{[S_t]}{[S_i]} = R;$$

(5)

$\tan \beta_i$ is obtained from observations on animals fully dark-adapted (Wolf and Crozier, 1927-28).

If the reformation of $S$ in the dark were a first order process, log $S_t$ should increase linearly with time. We do not know $S_t$ in absolute units, but we have a proportionate measure of it from (4) and (5). More precisely, if we assume $R = \frac{dx'/dt}{dx_t/df}$, where $dx'/dt = \frac{dx_t}{dt}$ is the rate of photolysis of $S$ when dark adaptation is at first complete, and therefore $\propto [S_\infty]$, then $-\frac{dS_t}{dt} = \frac{dx_t}{dt} = \frac{kS_\infty}{R}$; this, on the assumption of the moment, would be equal to $K_\infty (S_\infty - S_i)$, and log $\frac{S_\infty}{R'}$ or log $R$, should still be a linear function of time in the dark. The assumption of a “monomolecular” formation of $S$ can therefore be tested by plotting log $\frac{S_t}{R}$ or log $R$, against time of dark adaptation. As Fig. 3 shows, the relationship is certainly not rectilinear, and this assumption may be dismissed; moreover, it would leave unexplained the changes in slope of the curves of light adaptation.

If the formation of $S$ in the dark be assumed bimolecular, corresponding to

$$S \leftarrow P + A,$$

then $(S_\infty - S_t)$ must decrease hyperbolically with time in the dark,
or the graph of \( \frac{1}{(S\infty - S_t)} \) vs. \( t \) must be linear; but \( S\infty - S_t = (R - 1) \left( \frac{S\infty}{R} \right) \), and therefore \( \frac{1}{S\infty - S_t} = \frac{1}{S\infty} \cdot \frac{R}{(R - 1)} \); since \( \frac{1}{S\infty} \) is by assumption constant, the plot of \( \frac{R}{(R - 1)} \) against time in

![Graph showing dark adaptation in Agriolimax](image)

**Fig. 3.** If the course of dark adaptation were describable as a first order process log \( R \) should be linearly proportional to time in the dark,—which is obviously not the fact. \( R \) is the ratio between initial excitability after complete dark adaptation and that after \( t \) minutes in darkness. The excitability may be taken as proportional to \( [S] \), the concentration of photosensitive substance at the moment, or to \( \frac{ds}{dt} \).

darkness should show a linear increase. As is evident in Fig. 4, the relationship is anything but linear; in fact the curve shows a distinct sigmoid flexure and apparent relative inhibition of formation of \( S \) at the start. If we prefer to regard \( L \) as proportional to \( \frac{ds}{dt} \), then it is
easily shown that $R$, for a second order process, should be linearly proportional to $t$—which is certainly not the fact.

To account for the form of this curve it is necessary, as the simplest assumption, to invoke some type of autocatalysis. If the "dark" reaction were first order and autocatalyzed by $S$, then

$$\frac{dx}{dt} = K_1 (S_\infty - x) (x) + K' x$$

(6)

$$\frac{dx}{dt} = K_1 (S_\infty - x) (x) + K' x$$

Fig. 4. Were the course of dark adaptation susceptible to formulation as a second order reaction, then $\frac{R}{R - 1}$ should be a rectilinear function of confinement to darkness. Clearly, such formulation fails.

where $x$ = concentration of $A$ and of $P$; during exposure to light, also

$$\frac{dx}{dt} = K_1 (S_\infty - x), \text{ and}$$

$$\frac{ds}{dt} = K_4 (S_\infty - x) (x) + K' x - K_1 (S_\infty - x) = (K_5x - K_1) (S_\infty - x) + K' x$$

(7)

If for simplicity we assume, as has usually been done in the treatment of first order autocatalysis by a reaction product (cf. Crozier, 1926–27).
that $K'$ is negligibly small, then the course of dark adaptation ($K_1$ being then $= 0$) is given by

$$\frac{dx}{dt} = K_2 (S_\infty - x) \quad (x);$$

(8)

Fig. 5. The "dark" formation of photosensitive substance cannot be accounted for as the result of a first order process with positive autocatalysis, since log $\frac{x}{S_\infty - x}$ (or its proportionate equivalent, log $(R - 1)$), is not a linear function of time in the dark.

and, as is well known, the graph of log $\frac{x}{S_\infty - x}$ against time should show a linear decrease (if the value of $S_\infty$ is properly chosen); in terms of our present notation, from (4), log $\frac{x}{S_\infty - x} \equiv \log (R - 1)$. It is clear from Fig. 5 that the graph is not rectilinear; nor is its approach to rectilinearity at all improved by the assumption of other
(slightly different) magnitudes for \( S_o \). Moreover, even assuming for a proposed autocatalytic effect of first order type the more complete differential equation

\[
\frac{dx}{dt} = K_1 (S_o - x) (x) + K'x,
\]

the inflection point in the sigmoid curve relating \( S \) to \( t \) during dark adaptation must come at

\[
\frac{d^2S}{dt^2} = K_2S_o - 2K_1x + K' = 0,
\]

whence \( x = \frac{K_2S_o + K'}{2K_1} \); in other words, the inflection must come at a point such that \( S \) is less than \( \frac{1}{2} S_o \) (although it might be very close to \( S = \frac{1}{2} S_o \) ); but this quite disagrees with the facts (cf. Fig. 4, and Fig. 6). In terms of the more rigorous assumption that \( L \propto \frac{ds}{dt} \), the idea of first order autocatalysis is even more poorly substantiated.

V.

We must therefore give up a conception of first order formation of \( S \) during dark adaptation, and turn to a second order process (with auto-acceleration by \( S \)) as possibly giving the simplest description of the facts. The integral equations for such processes are not easy to handle, and indeed must be devised for the occasion; but they provide several decisive tests which are of considerable interest. These tests are of a nature which tends to remove one's suspicion that the agreement between formula and experiment might be merely a descriptively successful accident.

We will assume that the differential expression for the second order autocatalysis may be written

\[
\frac{ds}{dt} = K_4(S) (x^t) = K_1 (S_o - x) (x^t),
\]

neglecting, as before, the additional catalytic effect more properly
cared for by the introduction of another velocity constant. Then for the rate of change of \([S]\) in the light we will have

\[ - \frac{ds}{dt} = K_1 (S_m - x), \]

and for the dark reaction

\[ \frac{ds}{dt} = K_4 (S_m - S^2), \]

and therefore

\[ \frac{ds}{dt} = K_1 (S_m - x) (S^n) - K_1 (S_m - x), \quad (9) \]

or

\[ \frac{dx}{dt} = - \frac{ds}{dt} = K_4 S_m - K_4 x - K_4 S_m x^2 + K_4 x^2. \]

When \(x\) is very small (relatively complete dark adaptation) \(- \frac{ds}{dt}\) on exposure to light will thus depend largely on \(K_1 (S_m - x)\), and (other things equal) will be large (cf., Wolf and Crozier, 1927-28); when \(x\) is large \(- \frac{ds}{dt}\) will be low and its rate of change in light will be slowed, roughly in proportion to \(x^2\). This corresponds in a crude way to the increase in the slope of the curve of light adaptation as dark adaptation proceeds (Fig. 1).

The curve describing the change of photic excitability with increasing dark adaptation is given, on this assumption, by

\[ \gamma = - \frac{ds}{dt} = K_1 S_t - K_1 S_t (S_m - S)^n, \quad (10) \]

\[ = K_1 S_t - K_1 S_t S_m + 2 K_1 S_m S_t - K_1 S)^n, \]

where \(\gamma = - \frac{ds}{dt}\) is proportional to \(L\) at the first instant of exposure to the light, and so, as before, to \(1/R\). As \([S]\) increases, \(\frac{dy}{ds}\) is the slope of \(1/R\) plotted against \(S\) as \(S\) increases during the time of dark adapta-
tion. The slope of \(1/R\) passes through an inflection point, since \(dy/ds\) is maximum at

\[
d^2y/ds^2 = 0 = -4K_S + 6K_xS, \text{ or } S = \frac{1}{3}S_m,\]

whence

\[
ds/dt = \frac{ds}{dt} \cdot ds/dt / ds/dt = 1/R = \frac{1}{3}.
\]

![Graph showing the dark restitution of S through a second order reaction with positive autocatalysis](image)

**Fig. 6.** The "dark" restitution of \(S\) through a second order reaction with positive autocatalysis would require that the photic excitability, \(-ds/dt\), or its proportionate equivalent \(1/R\), should exhibit a maximum velocity of change when \([S]_0\) has been brought to a little less than \(\frac{1}{3} [S_m]\); \(1/R\) should therefore pass through an inflection point when \(1/R = 1\) a little less than 0.67 —; the inflection is found at \(1/R = 0.64\).
If the suppressed velocity constant \( K' \) be taken into account, \( x \) at the inflection point = \( \frac{1}{R} \left( \frac{K'}{K_2} + S_w \right) \) and \( \frac{1}{R} \) must be a little less than \( \frac{1}{3} \). This graph is given in Fig. 6. The general form of the curve is as expected, and the empirically determined inflection point is at about 0.63 or slightly more. The agreement is therefore strikingly good.

It might be said that the most sensible thing would be for the animal to be most rapidly dark-adapting when its receptors are most completely exhausted by light. But the natural behavior of these slugs is of course such that they are probably never driven normally to so complete a level of light adaptation as that involved in these experiments. Their negative heliotropism serves to carry them into dark places at sunrise, so that the most rapid phase of their dark adaptation in all probability corresponds to the maximum degree of light adaptation which they are called upon to experience. Even after hours of exposure to daylight (not direct sunlight) they are still negatively phototropic, but in a feeble way.

The sigmoid character of the course of dark adaptation is also made evident when the slugs are tested once after a measured period in darkness subsequent to light adaptation. The angles \( \beta \) are thus obtained for the first minute of exposure to light. Owing to the manner in which both intercept and slope of the lines in Fig. 1 change, the inflection must come earlier in the curve then relating \( \tan \beta_1 \) to time in darkness than in the curve in Fig. 6. The data for an experiment of this sort given in Fig. 7 are somewhat scattered, but the inflection is at about 15 minutes and thus definitely earlier than in Fig. 6.

The inflection point in Fig. 6 comes at about \( 1/R = 0.63 \), which corresponds to \( \beta = 44.5^\circ \). It would be reasonable then, to expect a greater variability in the observed values of \( \beta \) at about this magnitude of the orientation angle, since at this level of adaptation, with test light of this intensity, \( -\frac{ds}{dt} \) is changing most rapidly. Inspection of the probable errors (Table I) shows that the \( r.e. \) declines as \( \beta \) becomes less, but there are few measurements above \( \beta = 45^\circ \); in earlier series (Wolf and Crozier, 1927–28) the effect predicted is reasonably clear.
The integral for the curve of dark adaptation is obtainable only in an inconvenient form. Assuming that

\[ R = \frac{S_0}{S_t} \]

\[ \frac{ds}{dt} = K_s S_t (S_0 - S_t)^2 = \frac{K_s S_0}{R_0} (R - 1)^2 = -S_0 R^{3} \frac{dR}{dt}, \]

therefore

\[ -\frac{dR}{dt} = \frac{K_s S_0}{R} (R - 1)^2 \]

and

\[ -K_s S_0 \cdot t = \int \frac{RdR}{(R - 1)^2} = \log (R - 1) - \frac{1}{(R - 1)} + C, \]

or

\[ K_s S_0 (t_1 - t_0) = \frac{R_1 - R_t}{(R_1 - 1)(R_1 - 1)} + \log \left( \frac{R_1}{R_1 - 1} \right) \]

(11)

This enables us to evaluate \( K_s \) from the directly measured magnitudes of \( R \). The calculated values of \( K_s \), as obtained from equation (11) and the tabulated values of \( R \) (Table II), taking \( t_1 = 60 \) minutes dark adaptation, show satisfactory constancy (Table III). This reference-point is not the most accurately established, but permits computing from the most points. Values of \( R \) read from the smooth curve (Fig. 6) give better constancy for \( K'' \). Slight errors in obtaining \( R \) affect the computed value of \( K'' \) considerably. The systematic rise in \( K'' \) during the first 15 minutes in darkness might be explicable as due to the failure to consider the suppressed velocity constant in the autocatalytic formula (this makes \( \frac{ds}{dt} \) hence \( \frac{1}{R} \) appear "large"). Moreover, and this is most important, the derivation has assumed that the "dark" reaction is insignificant even at low concentrations of \( S \), which is inadequate, and results in \( K'' \) passing through a maximum.

The complete expression for the rate of light adaptation is even more unmanageable. But several of its properties may be deduced, which...
is all that is necessary for our purpose. If the conceptions underlying the analysis are correct, we must expect the rate of light adaptation to be a minimum when the rate of "dark" formation of $S$ is highest. The change in the slopes of the curves of light adaptation (Fig. 1)

### TABLE III.

Values of the velocity constant for dark adaptation, from $K_s S_m^a (t_1 - t_2) =$

$$\frac{R_2 - R_1}{(R_1 - 1) (R_2 - 1)} + \log \left(\frac{(R_1 - 1)}{(R_2 - 1)}\right),$$

using ascertained values of $R$ (Table II), taking $t_1 = 60$ minutes, and assuming $R = S_a/S_1$ (see text).

<table>
<thead>
<tr>
<th>$t_1 - t_2$ (min)</th>
<th>$K_s S_m^a = K''$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.320</td>
</tr>
<tr>
<td>30</td>
<td>0.341</td>
</tr>
<tr>
<td>35</td>
<td>0.358</td>
</tr>
<tr>
<td>40</td>
<td>0.314</td>
</tr>
<tr>
<td>45</td>
<td>0.294</td>
</tr>
<tr>
<td>50</td>
<td>0.267</td>
</tr>
<tr>
<td>55</td>
<td>0.241</td>
</tr>
</tbody>
</table>

should therefore supply an independent check on the interpretation. From equation (6),

$$- \frac{ds}{dt} = K_1 S_m^a - K_3 s - K_4 S_m^a s^2 + K s^3,$$

this should give a clue to singularities in the curve relating $- \frac{ds}{dt}$ to changes in the concentration of $s$, for which the slope is

$$- \frac{d^2 s}{dtds} = - K_1 - 2 K_3 S_m^a s + 3 K s^3.$$  \hspace{1cm} (12)

For a maximum or minimum the next differential must be equatable to zero, and for a minimum $- \frac{d^2 s}{dtds^2}$ must be positive.

$$- \frac{d^2 s}{dtds^2} = - 2 K_3 S_m^a + 6 K s^3.$$
therefore

\[ x = \frac{1}{3} S_m, \]

which corresponds to a minimum since

\[ -\frac{d^2 s}{d t^2} = 6K_s. \]

Fig. 7. The second order "dark" formation of \( S \) requires that the rate of light adaptation (i.e., change of \( -\frac{d s}{d t} \)) should be a minimum when \( \frac{d s}{d t} \) is increasing most rapidly. The minimum found shows precise agreement with this requirement.

The fuller formulation is

\[ -\frac{d s}{d t} = K_1 (S_m - z) - [K_2 + K_3(S_m - z)] x^3 \]

\[ = K_5 S_m - K_3 x - (K_3 + K_5 S_m) x^2 + K_4 x^3; \]

\[ \frac{d^2 s}{d t^2} = -K_1 - 2 (K_3 + K_5 S_m) x + 3K_4 x^2, \]

\[ \frac{d^2 s}{d t^2} = -2 (K_3 + K_5 S_m) + 6K_4 x; \]
at inflection,

\[
6K_x = 2(K_1 + K_3S_w)
\]

\[
x = \frac{1}{3} \left( \frac{K_1 + K_3S_w}{K_1} \right) = \frac{1}{3} \left( \frac{K_1 + S_w}{K_2} \right)
\]

therefore

\[
x = \text{a little} > \frac{1}{3} S_w.
\]

**Fig. 8.** A curve of dark adaptation as derived from single measurements of \( \beta \) with a number of slugs during the first minute of exposure to light subsequent to various periods in darkness following practically complete light adaptation. In this case, \( \beta \) corresponds to the values at 0.5 minute in Table I. Theory requires that the inflection of this graph should occur relatively earlier than in Fig. 2, which is the fact.

Hence the rate of light adaptation, with the test light of constant intensity, must pass through a minimum when \( x \) (i.e., \( S_w - S_i \),
or \( S_n (R - 1) \), has been reduced to slightly greater than \( \frac{1}{2} S_n \).

The minimum found (Fig. 7) is at \( t = 20 \) minutes or a little less; this corresponds precisely to the region of the inflection point in the previous curve (Fig. 6) describing formation of \( S \) in the dark, and indicates that the rate of light adaptation is least when the calculated

![Graph showing dark adaptation curves](image)

**Fig. 9.** Curves of dark adaptation as derived from repeated tests with the same individuals, at intervals during their photic adjustment in the dark. The shape of these graphs is to be contrasted with that in Fig. 8. The explanation is given in Fig. 10. (These curves were obtained from experiments at a temperature of about 15°, hence the time axis is extended by comparison with those earlier discussed.)

rate of formation of sensitive material is increasing most rapidly. The result is therefore quite reasonable.

Even if the tests for dark adaptation are made by the method of successive exposures of the same individuals to light during the progress of its dark adaptation, it is to be expected from the foregoing results that the change of the angle \( \beta \) should give a sigmoid curve when plotted against time. But since a certain amount of light
Fig. 10. The derivation of curves of dark adaptation of the two types obtained by the different procedures leading respectively to Fig. 8 and to Fig. 9. The fundamental curve of dark adaptation, that is of increasing initial excitability (0.5 minute in light), is given by the equations in the text or, graphically, by Fig. 6. Curve A is this graph. If taken at any point a on this curve and exposed to light, the excitability falls during the first minute of exposure to the level b, determined by the corresponding slope of the course of light adaptation at this level of adjustment (Fig. 1; Fig. 7). The magnitude of $1/R$ at point b may be gotten from such graphs as those in Fig. 8; or, in Fig. 1, from the ordinate intercepts at $t = 0.5$ minute (if the temperature and the intensity of the test light correspond; here, we are dealing only with the general shape of the resulting graphs). The curve of $1/R'$ is computed precisely in the manner of that for $1/R$, but on the basis of the ordinate intercepts (Fig. 1) at 0.5 minute exposure. On return to darkness, $1/R'$ begins again to increase, but now starts from level b. At intervals, this is all repeated. The continuous history deduced for $1/R'$ is then given by the dotted line. The observed change of excitability with time since first being placed in darkness should have the form given by the heavy line, which agrees with that found experimentally (Fig. 9); in the latter case the time axis is extended because the temperature was lower. The exact shape of curve B, particularly at the two ends, will depend on the frequency with which tests are made. There is thus a considerable interval over which the excitability does not change very much, when repetitive stimulation is employed. Curve C is curve B transformed to the same coordinates as Fig. 9.
adaptation is undergone during each interval of exposure, nothing
can be gotten directly from such a curve (Fig. 9) whereby to test the
quantitative formulation of the process. But it can be foretold that
the shape of the curve under these conditions ought to be significantly
different from that given in Fig. 7. Several curves obtained by
taking single readings of $\beta$ with slugs dark-adapted for different
intervals are given in Fig. 8. The direct synthesis of such curves is
explained in Fig. 10, without resort to the formulæ, and the result can
fairly be taken as a striking justification of the form of interpretation
here advanced.

VII.

The equation for the course of light adaptation as already given
(10) may be written (again neglecting the suppressed velocity con-
stant $K'$),

$$\frac{ds}{dt} = 2K_sS_wS_t^2 - K_sS_t + (K_1 - K_sS_w)S_t$$

or

$$-t = \int \frac{ds}{2K_sS_wS_t^2 - K_sS_t + (K_1 - K_sS_w)S_t}$$

whence

$$-t = \frac{1}{K_1 - K_sS_w} \left\{ \frac{1}{2} \log \frac{S_t}{(K_1 - K_sS_w) + 2K_sS_wS_t - K_sS_t} + \frac{K_sS_w}{\sqrt{K_1K_s}} \tanh^{-1} \frac{K_s(S_w - S_0)}{\sqrt{K_1K_s}} \right\} + C,$$

where $C$ is the constant of integration. In this case, $S_t = S_w$
when $t = 0$;

therefore

$$-t = \frac{1}{K_1 - K_sS_w} \left\{ \frac{1}{2} \log \frac{K_sS_t}{S_w(K_1 - K_sS_w) + 2K_sS_wS_t - 2K_sS_t} + \frac{K_sS_w}{\sqrt{K_1K_s}} \tanh^{-1} \frac{K_s(S_w - S_0)}{\sqrt{K_1K_s}} \right\} + C,$$  

(13)
Dark Adaptation in Agriolimax

At early stages of light adaptation, such as are measured in the curves in Fig. 1, beginning at any level of dark adaptation (S∞ − S) does not change greatly; and if K₂ is quite small, as it is known to be relative to K₁, then

\[ t = K''' \log S + K''', \text{ very nearly.} \]

It will be noticed that this is the relationship already illustrated in Fig. 1, although the linearity of the curve relating log tan β (≈, practically, to log S) to time did not enter into the nature of the data used in the subsequent deductions. Hence the conclusion now obtained from (11) is not to be regarded merely as "getting out something originally put into the formula."

VIII.

The present analysis of phototropic effects as influenced by adaptation is capable of being developed in several directions. We may mention, in addition to temperature relations, such points as: photic adaptation as affected by wave-length, and (possibly) polarization; dark adaptation subsequent to photic adaptation by light of particular wave-lengths as followed by means of recovery curves for excitation by light of other wave-lengths; and the changes of orientation when the geotropic vector is also varied. Experiments are being undertaken to explore these possibilities.

IX.

Summary.

A method is described which measures the excitation of Agriolimax by light, during the progress of light adaptation, by assuming that the orientating effect of continuous excitation is expressed as a directly

\[ \log \left( \frac{ds}{dt} \right) = \log [2K₂S∞S₁^2 - K₂S₂^2 + (K₁ - K₂S₂^2)S₁]; \]

if K₂ is very small, this is nearly equivalent to \( \log K₁S₁ \) for the same reason that the assumption of \( R = \frac{S∞}{S₁} \) is very nearly sufficient (cf. §V).

Because the same kind of result is obtained if the analysis be based merely upon the values of β during the first minute of exposure to light.
A proportionate tension difference in the orienting muscles of the two sides of the body. The tendency toward establishment of such a tension difference is caused to work against a similar geotropic effect at right angles to the phototropic one. This enables one to study the kinetics of light adaptation, and of dark adaptation as well. The situation in the receptors is adequately described by the paradigm

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

similar to that derived by Hecht for the differential sensitivity of various forms, but with the difference that the "dark" reaction is not only "bimolecular" but also autocatalysed by the reaction product $S$. The progress of dark adaptation is reflected (1) in the recovery of the amplitude of the orientation and (2) in the rates of light adaptation at different levels of the recovery; each independently supports these assumptions, for which the necessary equations have been provided. These equations also account for the relative variabilities of the angles of orientation, and, more significantly, for the two quite different kinds of curves of dark adaptation which are obtained in slightly different types of tests.

CITATIONS.


