PHOTOTAXIS OF Euglena gracilis

by

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ABSTRACT

Euglena gracilis is a single cell organism that exhibits phototaxis, that is, movement in response to a stimulus of light. This process appears to be controlled by a primitive visual system consisting of a photoreceptor and a shading device called an eyespot. An understanding of the physical and biochemical basis of phototaxis might help in understanding the fundamentals of more advanced visual systems. Using the phototaxigraph, an instrument for measuring phototaxis, some of the factors influencing this process have been investigated. The phototactic behavior of Euglena when exposed to polarized actinic light indicates that the eyespot carotenoid pigment molecules are aligned with their long axes parallel to the long axis of the organism and provides support for the shading role of the eyespot. A loss of phototaxis when Euglena are exposed to long periods of white light, and recovery of phototaxis after short exposure to high intensity far-red light, indicates the possibility of a photoreversible pigment system operating in the control of phototaxis. Experiments with pulsed actinic light show that the triggering and orienting influence of light in phototaxis take place in a time span of less than \( \frac{1}{2} \) second.
INTRODUCTION

Phototaxis in organisms can be defined as movement in response to a light stimulus. Movement toward the stimulating light is positive phototaxis and movement away from the stimulating light is negative phototaxis.

Members of the following groups of microorganisms are phototactic: purple photosynthetic bacteria, blue-green algae, diatoms and flagellated green algae. The last-mentioned group have specialized organelle systems which mediate the phototactic response and thus make convenient models for the study of sensory perception.

_Euglena gracilis_ is a single cell microorganism which has characteristics of both plants and animals. It is sometimes classified as an algae since it contains chlorophyll and is photosynthetic. _Euglena_ can also be classified as a protozoan inasmuch as it can grow heterotrophically in the dark, if a carbon source other than CO₂ is supplied. Under these conditions it does not develop a photosynthetic apparatus. _Euglena_ has an outer covering called a pellicle which is not a true plant cell wall. The organism is motile and generally exhibits positive phototaxis when exposed unilaterally to a light stimulus. Movement is accomplished by motion of a flagellum at the
anterior end which draws the organism through the medium. A pigmented organelle in the anterior end of *Euglena*, called an "eyespot," is thought to be involved in some manner in phototaxis. The present theory (Jennings, 1962; Batra and Tollin, 1964) is that the eyespot acts as a shading device for the true photoreceptor, which is a swelling at the base of the large flagellum (see Figure 1).

A phobo-phototactic response is a shock reaction mediated by a change in light intensity in which the direction of the light rays is not thought to be involved. For example, the purple photosynthetic bacterium *Rhodospirillum rubrum* can be trapped in a spot of light, since the bacterium reverses its direction when it swims out of the zone of light. The bacteria are thus prevented from leaving the spot of light but not from entering it. They have a phobic response to a decrease in light intensity but are not attracted to the light. This would be defined as a positive response; a negative response would be the inverse of this.

A topo-phototactic response is directed movement with respect to the position of a source of light. *Euglena* rotates as it swims, so that in the presence of unilateral illumination the photoreceptor at the base of the flagellum will be shaded periodically by the eyespot. It has been
Figure 1. Schematic Drawing of *Euglena gracilis*
suggested (Jennings, 1962) that this shading causes a succession of phobic reactions which act to point *Euglena* toward the source of light. When the cell is moving toward the light, the shading of the photoreceptor occurs* and there is no phobic response. This permits a continuation of movement in the same direction. In this way, the combination of eyespot and photoreceptor would act as a servomechanism in the orientation of *Euglena* towards the light.

An instrument, called a phototaxigraph, which can measure rates and extents of phototaxis, was developed by Lindes, Diehn and Tollin (1966) and was available for use on this project. The phototaxigraph records the change in optical density as organisms accumulate within the light stimulated zone of the *Euglena* suspension, as compared with an unstimulated region of the suspension (see Figures 2 and 3). In the recording of Figure 3, the ordinate is optical density and the abscissa is time (going from right to left). After the light is turned on, there is about a 15 second lag before an increase in optical density is recorded. Then there is a steady increase in optical density.

*It is also possible that the inverse is true, i.e. that the shock response occurs upon shading the photoreceptor. However, there is some evidence against this type of mechanism, e.g. negative phototaxis in eyespotless *Euglena* (Diehn and Tollin, 1966b) and polarized light effects (see below).*
FIGURE 2: OPTICAL CONFIGURATION OF THE PHOTOTAXIGRAPH
Figure 3. A Typical Phototaxigram
(corresponding to accumulation of organisms within the illuminated zone) until about 20 seconds after the light is turned off. The Euglena then begin to disperse and the curve drops back down to the baseline after several minutes.

The following points about phototaxis in Euglena were determined by Diehn and Tollin (1966a, b, 1967) using the phototaxigraph: (1) There are diurnal variations of phototaxis of Euglena. The rate of phototaxis is greatest in the first 6 hours of the light period and decreases to a low point after about 6 hours in the dark, when the organisms are grown on a 12 hours light - 12 hours dark cycle. (2) A lag time of about 15 seconds is present after the light is turned on during which no accumulation of Euglena in the stimulated zone is recorded. Under some conditions, a similar lag time is seen after the light is turned off, during which the organisms continue to accumulate. The lag times are factors in the response of the organisms rather than an instrumental artifact. (3) 25°C is the optimum temperature for phototaxis in Euglena, with a decrease in rate on either side of the optimum. (4) It appears that it is necessary for photosynthesis to be operating for phototaxis to occur. Probably, the energy for phototactic orientation derives from photophosphorylation. A continuation of phototactic ability for a period of several hours after inhibition of photosynthesis might be due to a pool of substance built up for
phototaxis by photosynthesis. (5) The action spectrum for phototaxis of *Euglena* has a peak at 460 m$\mu$ and drops sharply at either side to give zero phototaxis at 650 m$\mu$ and at 350 m$\mu$. This agrees with the eyespot absorption spectrum and is thus consistent with the postulated shading role of this organelle.
MATERIALS AND METHODS

*Euglena gracilis*, strain z was originally obtained from the University of Indiana, Algae Collection, Bloomington, Indiana. The original medium used (designated DL Medium) contained the following materials:

<table>
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<tr>
<td>(NH₄)₂ HPO₄</td>
<td>10</td>
</tr>
<tr>
<td>KH₂ PO₄</td>
<td>10</td>
</tr>
<tr>
<td>Mg SO₄</td>
<td>5</td>
</tr>
<tr>
<td>Ca Cl₂</td>
<td>0.8</td>
</tr>
<tr>
<td>DL Malic Acid</td>
<td>15</td>
</tr>
<tr>
<td>Metals Mix (Bach, 1950)</td>
<td>1 ml/l</td>
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<tr>
<td>Vitamin B₁₂</td>
<td>4 μg/l</td>
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The DL Medium was used in the polarized light experiments. In the pulsed light and far-red irradiation experiments, the lactic-acid rapid growth medium described by Wolken (1967) was used. Organisms grown in the latter medium generally gave better phototactic responses.

The cultures were grown in an incubator maintained at 25°C. The normal lighting conditions were alternating light (100 ft. candles, fluorescent) and dark periods of 12 hours duration each, beginning at 6 a.m. and 6 p.m. respectively.
Euglena suspensions of the same initial optical density \(O.D._{800} = 0.3\) were used in all of the phototaxis runs.

In the pulsed light experiments, the light intensity was controlled using perforated metal screens as neutral density filters. The duration of light and dark pulses was controlled using Heathkit electronic timers to operate a solenoid shutter.

In the far-red irradiation experiments, the far-red light was obtained by using a Corning infrared transmitting, visible absorbing filter (color specification 7-69), which gives no transmittance below 720 \(\text{nm}\) and has a transmittance range of 720 \(\rightarrow\) 1000 \(\text{nm}\). Bausch and Lomb interference filters were used for the 660 \(\text{nm}\) and 630 \(\text{nm}\) wavelengths. The light source was a 500 watt tungsten filament projection lamp. Infrared radiation was removed using a water cell (3 cm) and several heat-absorbing filters.

Polarized light was obtained by placing a piece of Polaroid in the actinic beam.
RESULTS AND DISCUSSION

Effect of Pulsed Actinic Light on Phototaxis

In the introductory chapter, reference was made to the existence of on and off lags in the phototaxis response curves. In order to further investigate the cause of the lag times and to determine minimum phototactic response times of *Euglena*, timers were used with the phototaxigraph to give varying light and dark pulses during a phototaxis run. Figure 4 shows a phototaxigram obtained using pulsed stimulation of 40 seconds light and 40 seconds dark. It can be seen quite clearly that there is a 10-15 second lag time between application of a light stimulus and the beginning of phototactic accumulation (on lag), and about a 20 second lag time between removal of the stimulus and the cessation of accumulation (off lag). The fact that we observe smooth curves with no discontinuities indicates that shock reactions to the turning of the light on or off probably do not play a major role in causing the lag phenomena. Similarly shaped curves have been obtained with a variety of other light and dark times.

The inserts at the left of Figure 4 show phototaxigrams obtained with single light pulses of $\frac{1}{2}$ second and 1
Figure 4. Effect of Light-Dark Pulses and Short Duration Light Pulses on Phototaxis

(a) 40 second light and dark pulses, (b) single ½ second light pulse, (c) single 1 second light pulse
second respectively. It can be seen that a light pulse of \( \frac{1}{2} \) second duration is detected by the Euglena and produces a phototactic response.

A series of phototaxis runs were made in which continuous rapid light and dark pulses were used. The purpose of these experiments was again to investigate the role of negative shock reactions to the light and also to determine the efficiency with which short light pulses elicit phototaxis. In Figure 5 a normal run with 1% minutes of light (a) is compared with a curve obtained using repeating \( \frac{1}{2} \) second light and 1 second dark pulses (b). It is seen that the curve obtained with the light-dark pulses rises somewhat more slowly than the normal curve and shows a rapid increase in accumulation immediately after the pulsing light is turned off. The on lag, however, is about the same as for the normal run, again indicating a small role of shock reactions in this phenomenon. The simplest explanation for the enhanced accumulation after discontinuing the light stimulus is that the pulsed light produces a negative shock reaction which is stopped when the pulses cease. The slower rate of accumulation is produced partly by the shock reaction to the light pulses and partly by a decrease in the total amount of light which the Euglena are exposed to.
Figure 5. Effect of $\frac{1}{2}$ Second Light-$1$ Second Dark Pulses on Phototaxis

(a) $1\frac{1}{2}$ minutes light, (b) $\frac{1}{2}$ second light-$1$ second dark pulses
Another series was run with pulses of 1 second light and 1 second dark. It can be seen in Figure 6a and c that the light pulses still produce a negative shock reaction which shows up as an enhancement in accumulation after the light is turned off. However, this enhancement is not as pronounced as in Figure 5, where \( \frac{1}{2} \) second light pulses are being used. Therefore, the increased duration of the light pulse has decreased the negative shock reaction to the light. It is interesting that the lower intensity of actinic light in Figure 6c does not cause a decrease in the extent of the shock reaction.

In Figure 7 which shows curves obtained using pulses of 3 second light and 1 second dark, it is seen that the enhancement of accumulation on turning the light off is hardly noticeable. Thus, within three seconds after the light has been turned on, the Euglena have become adapted to the light and are not experiencing a shock reaction. In the initial 10-15 second on lag during a normal phototaxis run it would appear that only about 2 seconds can be attributed to a negative shock reaction to the light. Other factors, such as the inertia inherent in the swimming motion of the Euglena, must be contributing to the major portion of this on lag time.

It was shown in Figure 4 that a single \( \frac{1}{2} \) second pulse of light is sufficient to produce a phototactic
Figure 6. Effect of 1 second light-1 second dark pulses on phototaxis

(a) 1 second light-1 second dark, no filter; (b) 2 minutes light, 0.3 neutral density filter; (c) 1 second light-1 second dark, 0.3 neutral density filter.
Figure 6. Effect of 1 second light-1 second dark pulses on phototaxis
Figure 7. Effect of 3 Second Light-1 Second Dark Pulses on Phototaxis

(a) 3 second light-1 second dark, no filter; (b) 2 minutes light-0.77 neutral density filter; (c) 3 seconds light-1 second dark, 0.77 neutral density filter.
Figure 7
response in *Euglena*. A comparison of the two curves in Figure 5 shows that a series of $\frac{1}{2}$ second pulses produces almost as great a response as an equal duration of continuous light. Similarly, if one compares the curves in Figure 5b, Figure 6c and Figure 7c, in which neutral density filters were used to keep the total amount of light reaching the organisms constant, it is apparent that *Euglena* can respond about as well to $\frac{1}{2}$ second pulses as to 1 or 3 second pulses. It is important to point out that the results using continuous stimulating light of varying intensity (Figures 5a, 6b and 7b) demonstrate that we are not operating at saturating light intensities. The smaller response in Figure 7c, as compared with Figures 5b and 6c, is probably due to the fact that the light intensity dependence of phototaxis is logarithmic rather than linear (Diehn and Tollin, 1966a).

These results demonstrate quite clearly that the actinic light is the triggering and orienting stimulus for phototaxis but not the direct source of energy (Diehn and Tollin, 1966a). The facts that the $\frac{1}{2}$ second pulses of light are about as effective as continuous light and that a single $\frac{1}{2}$ second light pulse is sufficient to produce a phototactic response show that this triggering and orienting influence can take place in a time span of less
than $\frac{1}{2}$ second. With the present equipment light pulses of less than $\frac{1}{2}$ second could not be produced or recorded accurately.

Phototactic Response of Euglena to Polarized Light

Evidence had been found earlier (Wolken, 1961) that Euglena have a greater motility in polarized light than in unpolarized light. Many species of animals, mostly arthropods such as the crab Cardisoma have been shown to be capable of detecting and responding to linearly polarized light (Waterman, 1966). It has also been found that, when exposed to polarized light, germinating spores of the fungus Botrytis and the fern Osmunda orient their growth relative to the plane of polarization (Jaffe, et al. 1962). To test the effect of polarized light on Euglena phototaxis, a Polaroid filter was inserted in the phototaxigraph to give plane polarized light in the actinic beam. One plane of polarization produced the same shape phototaxigram as did unpolarized light (see Figure 8b and c). However, when the plane of polarization was rotated $90^\circ$ to this plane, a distinct difference in behavior of the Euglena was produced (see Figure 8a). Intermediate positions of the polarizer produced phototaxigrams of intermediate shape.
Figure 8. Comparisons of phototaxigrams obtained using actinic polarized light and actinic unpolarized light

(a) polarized light e-vector parallel to axis of rotation of tube; (b) unpolarized light; (c) polarized light e-vector perpendicular to axis of rotation of tube.
Figure 8. Comparisons of Phototaxigrams obtained using Actinic Polarized Light and Actinic Unpolarized Light
The characteristic features of the polarized light effect are an initial negative phototaxis occurring immediately when the light is turned on and a sudden increase in density of *Euglena* in the actinic zone when the light is turned off.

We had difficulty in interpreting the polarized light effect because of our belief that the *Euglena* were randomly oriented at the time that the actinic light was turned on. Without any orientation of the *Euglena*, a mechanism for different responses to different planes of polarized light did not seem obvious. Therefore, we examined our system to see if there was some orientation of organisms in a preferred direction prior to turning on the actinic light. In the phototaxigraph, the sample tube is rotated at 10rpm to keep the *Euglena* from settling and to expose all sides of the tube to the same amount of light. Photomicrographs of the rotating and non-rotating tube were taken and are shown in Figure 9. It can be seen that when the tube is not rotating the organisms are randomly oriented, but when the tube is rotated the organisms are mostly aligned perpendicular to the axis of rotation. This is in agreement with the observation that free-swimming protozoa orient themselves against a current (Kudo, 1966).

The finding that the *Euglena* are oriented, and the determination of their direction of orientation with relation
Figure 9. Photomicrographs of Euglena gracilis in a Non-Rotating (a) and a Rotating Tube (b) (X100)
to the plane of polarization of the actinic light, allows not only an interpretation of the phototactic behavior but also gives a clue as to the molecular geometry of the eyespot. The present theory of phototaxis is that the eyespot acts as a shading device for the photoreceptor at the base of the flagellum. We assume that the Euglena orient themselves while swimming so that the photoreceptor is shaded from the source of light by the eyespot. If one also assumes that the pigment molecules in the eyespot are oriented with respect to the plane of the organism, then the initial negative phototaxis can be interpreted as a shock reaction produced when the light which is polarized in such a manner so as not to be absorbed by the eyespot pigments (i.e. so that the plane of polarization is perpendicular to the molecular electronic transition moment) illuminates the photoreceptor. This shock reaction is experienced by those Euglena directly in the actinic beam and causes a decrease in the number of organisms within the actinic zone, probably by causing a reversal of swimming direction at the dark-light interface. This type of response would be similar to that observed with eyespotless mutants (Diehn and Tollin, 1966b).

The Euglena outside of the actinic zone are exposed to scattered light which has lost a good deal of its polarized character. Therefore, as shown in Figure 8, after an
initial period of negative phototaxis caused by the shock reaction, a positive slope corresponding to positive phototaxis is observed.

As explained in the preceding section on the effect of light pulses on phototaxis, there is normally a 15 second lag time after the unpolarized actinic light is turned off, during which the Euglena will continue swimming toward where the light was. With polarized actinic light, as in Figure 8a, the rate of accumulation in the actinic zone is much greater than with unpolarized actinic light for about 15 seconds after the light is turned off. This is probably produced by a combination of the normal inertial lag and a cessation of the opposing shock reaction to polarized light.

The clue to the geometry of the pigment molecules in the eyespot is provided by the fact that only the light polarized in a plane parallel to the axis of rotation of the sample tube produces the negative shock reaction. This plane of polarized light is perpendicular to the long axis of the aligned Euglena. Therefore, the pigment molecules must be aligned in the eyespot so that the molecular transition moments are parallel to the long axis of the organism, as shown in Figure 10. The pigment molecules in the eyespot are known to be carotenoids (Batra and Tollin, 1964). The molecular transition moment of carotenoids
Figure 10. Diagram Showing Probable Orientation of Eyespot Pigments in *Euglena*
is parallel to the plane of the extended conjugated double bonds. Therefore, the carotenoids in the eyespot of *Euglena* are most likely arranged with the long axes of the molecules parallel to the long axis of the organism.

**Enhancement of Phototaxis by Far-Red Irradiation**

*Euglena gracilis* loses its phototactic response when kept under continuous light for 24 hours (Diehn and Tollin, 1966a). Recovery of phototaxis is complete after a normal cycle of 12 hours dark and 12 hours light. After 24 hours of continuous light only about 30% of the *Euglena* are motile. After 2 hours of dark, the motility is almost completely regained. However, the phototactic ability has not been recovered at all.

In attempting to find the cause of this loss and recovery of phototaxis, the possibility of a photoreversible pigment system operating in the control of phototactic ability was investigated. The type of system which occurred to us was one analogous to the phytochrome system in higher plants. In these organisms, many aspects of growth and response are controlled by a reversible photoconversion of two forms of a blue chromoprotein called phytochrome (Hendricks, 1964). The conversion is:

\[
\text{Phytochrome} \xrightarrow[660 \text{ m} \mu]{} \text{Phytochrome} \xrightarrow[730 \text{ m} \mu]{} \text{Phytochrome} \xrightarrow{\text{Dark}} \text{Phytochrome} \xrightarrow[660 \text{ m} \mu]{}
\]
Here 660 mμ and 730 mμ are the respective absorption maxima of the two forms of phytochrome. Flowering, stem elongation, leaf movement and seed germination are among the higher plant responses that are controlled by this system.

In Euglena kept under continuous light, such a pigment might undergo a photoreaction producing a form ineffective in causing phototactic ability to develop. If such was the case, by irradiating with the proper wavelength to convert the pigment to the active form for phototaxis, a rapid recovery of phototaxis might be obtained. Euglena suspensions which were taken immediately after 24 hours of continuous light and exposed to irradiation of far-red light, using a filter with no transmission below 720 mμ, showed no enhancement of phototaxis. Far-red exposures at high intensity ranging from 5 minutes to 1 hour were used. However, when samples were used that had been exposed to 24 hours of continuous light and then were dark-adapted for 2 hours, a considerable enhancement was obtained, as shown in Figure 11. It is not clear at present why the period of dark adaptation is required, but it may be related to the recovery of motility. Five minutes of high intensity far-red irradiation causes little or no enhancement; 10 minutes of irradiation causes enhancement of the degree shown in Figure 11. Further far-red irradiation, for up to ½ hour, causes no significant change.
Figure 11. Enhancement of Phototaxis by Far-Red Irradiation

(a) phototaxis curve obtained after 10 minute far-red irradiation of dark-adapted sample used in b, (b) dark-adapted *Euglena*
in the degree of enhancement. No observable changes in motility were produced by the far-red irradiations.

Attempts to reverse the enhancement by irradiation at 660 m\(\mu\) and at 630 m\(\mu\) were unsuccessful.

No effect of far-red irradiation was observed with normally-grown cultures.
CONCLUSIONS

Our interpretation of the polarized actinic light effect gives additional support to the theory that the eyespot acts as a shading device for a photoreceptor which lies below it, presumably at the base of the flagellum. The results also indicate that the pigment molecules in the eyespot are aligned parallel to the long axis of the organism. The fact of pigment molecule organization is reminiscent of the situation in the retina (Denton, 1954) and the chloroplast (Calvin, 1959).

If our theory is correct that the polarized light which produces a negative phototactic response is not screened by the eyespot pigments then an action spectrum of this effect should give the absorption spectrum of the photoreceptor pigment with little or no distortion caused by eyespot pigments.

In the section on pulsed light effects it was shown that the triggering and orienting effect of light for phototaxis takes place in a time span of much less than \( \frac{1}{2} \) second. \textit{Euglena} requires about 1 second to complete a full revolution about its long axis (Jahn, 1964). Thus, considerably less than one full rotation, on the average, must be necessary in order for at least some of the \textit{Euglena}\n
30
(perhaps only those which are favorably oriented) to sense the direction of the light. Since accumulation of organisms proceeds for 10-20 seconds after exposure to a single \( \frac{1}{2} \) second pulse, the directional information must be "stored" for this length of time. Perhaps part of this "storage" is merely the inertia inherent in the swimming motion of the Euglena, i.e. once they start swimming in a given direction, as a result of the signal provided by the light pulse, they continue for 10-20 seconds in that same direction.

The recovery of phototaxis, caused by irradiation with far-red light, in Euglena kept in the light for over 24 hours and then dark-adapted, is suggestive of a photo-reversible pigment system operating in the control of phototaxis. However, a key experiment, which was attempted but was not successful, would have been to cause a decrease in the extent of phototaxis in a sample by irradiating at some particular wavelength range. This would have completed the analogy with the higher plant systems. The wavelengths tried were 660 m\( \mu \) and 630 m\( \mu \) but these had no effect on the extent of phototaxis. A possible reason for this inability to reverse the enhancement effect may be that the substrates for the active form of the pigment are in such abundance that they are triggered into action and continue to act even though the initiating pigment has been photoconverted to its inactive form. Therefore, photo-reversal might be exhibited only when there is a time
limitation imposed on the phototaxis by a limited substrate supply. It would be worthwhile exploring this further. Also, it would be of interest to obtain an action spectrum for the far-red enhancement.
LITERATURE CITED


