Photoinhibition of Growth in Acanthamoeba castellanii Cultures

WARREN D. DOLPHIN

Faculty of Microbial and Cellular Biology, The Ohio State University, Columbus, Ohio 43210

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Light from 350 to 680 nm at intensities up to \(1.62 \times 10^4\) ergs per sec per cm\(^2\) slowed exponential growth and lowered the maximum yield in axenic cultures of Acanthamoeba castellanii. Photoinhibition was a linear function of light intensity up to \(1.25 \times 10^4\) ergs per sec per cm\(^2\). At higher intensities, growth was too slow to be measured accurately. A photochemical change occurring in the growth medium on irradiation was a function of light dosage and not intensity per se. Light in dosages which appreciably changed the growth-supporting properties of the medium exceeded the dosages received by exponentially growing cultures during irradiation. Consequently, photoinhibition of growth was attributed to a direct effect of light on the amoebae, not to photodegradation of the medium. The growth-supporting properties of irradiated media could be restored by the addition of yeast extract and Proteose peptone. The reduced growth rate in the light was not due to cyst formation or induction of multinuclearity. Light affected the amoebae either by absorption by intracellular pigment(s) or through binding to the amoebae of a photosensitizing compound in the medium.

Among protozoa, photoinhibition of growth has been shown in cultures of Paramecium (9, 20), Tetrahymena (7, 16), Blepharisma (8), and in bleached phytoflagellates (3). The response of sarcodinid cultures to light has not been reported except for some observations of phototaxis and cytokinesis by Prescott (18) and by my preliminary reports (4, 5).

This paper describes the changes occurring when cultures of Acanthamoeba castellanii are irradiated with visible light. Evidence is presented that allows the interaction of light with the amoebae and the culture medium to be separated into two distinct effects. The effect of light on the amoebae suggests future work on the molecular mechanisms of the observed photoinhibition.

MATERIALS AND METHODS

Culturing and sampling. A. castellanii, strain I-12, was grown axenically on Neff's optimal growth medium [OGM; reference 14, see Byers et al. (2) for details of medium preparation]. Stock cultures in late-exponential growth were diluted to ca. 1,000 amoebae/ml, and 3-ml portions were pipetted into screw-top culture tubes (15 by 125 mm). These tubes were incubated at 30°C under various light intensities in the apparatus diagrammed in Fig. 1. When OGM was irradiated before adding cells, the same type of tube was used as in the growth studies. After pre-irradiation, the OGM was collected, inoculated, and redistributed into tubes which were incubated in the dark.

At each sampling time, three culture tubes were taken, the amoebae were fixed by adding a drop of Formalin, and cell concentrations were determined with an electronic counter (Celsoscope, Particle Data Co., Elmhurst, Ill.). The mean cell density obtained at each sampling time was used to plot the growth curves. For the linear exponential portion of the growth curves, a least-squares analysis was used to calculate the slope of the regression line. This was used to determine the population doubling time and the normalizing time, a value added to each sampling time to make zero time of the growth curve correspond to 1,000 amoebae/ml.

Irradiation apparatus. A 1,500-W "Quartzline" lamp (GE model Q150073/CL), mounted in a parabolic reflector (GE C522G005) and operated at 240 V, served as a light source (Fig. 1). Directly beneath the light housing, a water tank (7.5 cm deep) acted as a preliminary infrared filter, removing wavelengths above 980 nm (6). The bottom of the tank was a "pebbled-design" glass which diffused light over the tube bed below. Directly beneath the water filter was a second glass tank filled with 0.64% CuSO\(_4\) to a depth of 10 cm. This filter further reduced the transmitted spectrum by absorbing radiation above 680 nm (19). The filter combination lessened the danger of greenhouse effects which would have raised the temperature inside the culture tubes. With the de-
scribed apparatus, the internal temperature was maintained at 30 ± 0.5 °C.

Light intensities were measured with a thermopile radiometer (Yellow Springs Instrument Co.) and were constant (±5%) over the tube bed area. Light intensity was varied by moving the lamp housing, moving the water-bath jack, or interposing layers of cheesecloth between lamp and culture tubes.

The light energy spectrum at the surface of the culture tubes was determined with the radiometer and narrow-band (10 nm) interference filters (Oriel Optics Co.; Fig. 2A). The lower wavelength limit was determined by the output of the lamp and the upper by the transmission of the CuSO₄ filter. Curve A represents the maximum light energy which could reach the cells, and curve B the minimum. Curve B was constructed by correcting curve A for the transmission properties of the OGM and of Pyrex glass (14). The quality of the light reaching the cells cannot be specified except within these limits, because the culture tubes were slanted and the medium depth was not constant. Also, as the cell density increased, mutual shading probably lowered the amount of light reaching each cell. Curve C depicts the spectral distribution of the energy of sunlight on a bright day and is included for comparative purposes. The intensity in a room illuminated by incandescent lamps would barely be above the baseline in this figure.

![Figure 1. Irradiation apparatus.](image)

**FIG. 2.** Curve A, spectral distribution of energy from irradiation apparatus at a total intensity of 1.55 × 10⁶ ergs per sec per cm². Curve B, minimal amount of energy which could be received by cells with light at intensity as in curve A. The differences between A and B are due to the absorption of light by culture tube walls and OGM. Curve C depicts solar spectral energy distribution on a bright day (see reference 13).

Cyst-to-trophozoite ratios and nuclear number counts. Cyst-to-trophozoite ratios were calculated from a differential count made of at least 1,000 cells by using phase-contrast optics and a Fuchs-Rosenthal hemocytometer slide. The average number of nuclei per cell was determined with the techniques of James and Byers (10), except that a slide with concentric circles etched on it was used in place of one with a spiral.

**RESULTS**

Growth in continuously irradiated cultures. Figure 3 illustrates the growth curves of four cultures continuously irradiated at different light intensities. The population growth rate and the maximum cell concentration achieved in a culture were reduced by increasing irradiation intensity. At the highest light intensity (D), growth was almost completely inhibited. In Fig. 4, the relative increase in the doubling time is plotted as a function of the intensity of irradiation up to 1.25 × 10⁶ ergs/sec/cm². The slope is equivalent to an increase of 0.69 hr over the doubling time in the dark (9.8 hr) for each 10⁴ ergs/sec/cm² increase in the irradiation intensity.

Several hypotheses were made to explain the
in irradiated and became slowly dividing to the component sensitive. The if or photosensitized by photochemical reaction destroyed inhibition. Light would increase population doubling time if mononucleates were converted to slowly dividing multinucleates through inhibition of cytokinesis, if multiplying trophozoites encysted and became nonmultiplying cysts, if a photochemical reaction destroyed an essential component of the OGM, if the amoebae were photosensitized by a component of the medium, or if a rate-limiting cellular process was photosensitive. The following data limit the alternatives.

**Nuclear number and cyst-to-trophozoite ratios in irradiated cultures.** James and Byers (10) reported that induction of multinucleates in Acanthamoeba cultures is accompanied by decreased growth rate. Light can cause the formation of binucleate cells in cultures of Amoeba proteus (18). When the number of nuclei per cell in irradiated and control cultures was counted, there was no increase in the number of nuclei per cell in response to irradiation (Table 1).

Table 2 lists the cyst-to-trophozoite ratios measured at the same time as the nuclear number. During the exponential phase of growth at all light intensities, the number of cysts was low and relatively constant. As the cultures went into stationary phase, the ratio increased but there was no differential effect due to irradiation.

**Growth in previously irradiated medium.** To determine whether a growth-limiting photochemical change occurred in the OGM, it was irradiated before adding cells and then inoculated, and the cultures were grown in the dark. In Fig. 5, curves A, B, C, and D represent populations in medium irradiated for 0, 24, 93, and 117 hr, respectively, at an average intensity of $1.13 \times 10^4$ ergs/sec/cm². Increasing exposure of the OGM before adding amoebae decreased its growth-supporting properties and the maximum cell density. Figure 6 illustrates the increase in the doubling times of populations grown in pre-

![Figure 3](image-url)  
**FIG. 3.** Growth curves of cultures irradiated at four light intensities. Curve A represents a population growing in the dark. Curves B, C, and D represent cultures irradiated at $4.5 \times 10^4$, $1.25 \times 10^4$, and $1.62 \times 10^4$ ergs per sec per cm², respectively. $T_d =$ time for population to double in number during exponential growth.

![Figure 4](image-url)  
**FIG. 4.** Relative increase in population doubling time as a function of irradiation intensity.

**TABLE 1. Average number of nuclei per cell at three light intensities**

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>Cell concn</th>
<th>Light intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>Initial</td>
<td>$1 \times 10^3$</td>
<td>1.010</td>
</tr>
<tr>
<td>Early-exponential</td>
<td>$5 \times 10^3$</td>
<td>1.006</td>
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<tr>
<td>Mid-exponential</td>
<td>$2 \times 10^4$</td>
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</tr>
<tr>
<td>Late-exponential</td>
<td>$1 \times 10^4$</td>
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<tr>
<td>Decelerating</td>
<td>$3 \times 10^4$</td>
<td>1.020</td>
</tr>
<tr>
<td>Stationary</td>
<td>$1 \times 10^6$</td>
<td>1.092</td>
</tr>
</tbody>
</table>
TABLE 2. Cyst-trophozoite ratios at three light intensities

<table>
<thead>
<tr>
<th>Growth phase</th>
<th>Cell concn</th>
<th>Light intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dark</td>
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<tr>
<td>Initial............</td>
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</tr>
<tr>
<td>Early exponential</td>
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<td>0.002</td>
</tr>
<tr>
<td>Mid-exponential</td>
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<td>0.001</td>
</tr>
<tr>
<td>Late-exponential</td>
<td>$1 \times 10^4$</td>
<td>0.001</td>
</tr>
<tr>
<td>Decelerating</td>
<td>$3 \times 10^4$</td>
<td>0.001</td>
</tr>
<tr>
<td>Stationary........</td>
<td>$1 \times 10^4$</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Vitamin $B_12$ and thiamine are unstable in light. However, when added to preirradiated media they failed to restore normal growth, indicating that some other material was destroyed by light.

Proteose peptone (PP) or the yeast extract (YE) were added individually and in combination to medium which had been preirradiated at $10^{11}$ ergs/cm$^2$ and to nonirradiated medium. These media were then inoculated and the growth of the populations was traced in the dark (Fig. 7). The populations in all the nonirradiated media had a generation time of 7.8 hr, whereas those in the irradiated, nonsupplemented medium had a generation time of 30.3 hr. The irradiated, supplemented media displayed a differential response depending on the supplement. The addition of YE or PP alone greatly reduced the generation time in the irradiated cultures; however, neither fully restored the doubling times to that of the control. The addition of YE and PP together, however, brought the doubling time back to essentially that of the control. The effects of the supplements on the maximum cell density in irradiated medium are not shown in Fig. 7. The addition of YE or PP tended to restore the yield. However, when YE and PP both were added, the yield was slightly greater than in the nonirradiated control.

Light pulse studies. To test in one culture

irradiated medium to normal, it was the photolabile component.

Medium supplement studies. The photolabile component of the medium was inferred from experiments in which preirradiated OGM was supplemented with the normal components of the medium, one at a time, after irradiation but before inoculation. The rationale was that if a supplement restored the growth rate in the pre-

Fig. 5. Growth of cultures in the dark following preirradiation of the media at four light dosages. Curve $A$, control 0.0 ergs/cm$^2$; curve $B$, $1.09 \times 10^6$ ergs/cm$^2$; curve $C$, $3.42 \times 10^6$ ergs/cm$^2$; curve $D$, $5.48 \times 10^6$ ergs/cm$^2$.

Fig. 6. Relative increase in population doubling time as a function of the dosage which the medium receives before additon of amoebae.
PHOTOINHIBITION OF _ACANTHAMOEBA_ GROWTH


whether the photorepression of growth observed in the original irradiations was partly due to an effect directly on the cells or whether it was due entirely to the photochemical changes in the OGM, a dark-grown population was given a light pulse at mid-exponential phase (Fig. 8). During the light pulse, the growth rate in these cultures was reduced but increased almost to that of the control 3 hr after the end of the light period. The doubling time on return to dark conditions, however, was slightly greater than the control. The difference was equal to the increase in doubling time expected in medium which had been irradiated with an equivalent dosage before inoculation. This indicates that light affects the cells directly and that the photodegradation of the OGM is not a sufficient explanation for the total inhibition observed in continuously irradiated cultures.

_Pigments in Acanthamoeba_. If light directly affects the amoebae, they must possess an endogenous pigment. Page (16) observed yellow bodies in the cytoplasm of _Acanthamoeba_. When amoebae are packed in a pellet in a centrifuge tube they appear brownish. A pigment was extracted from these pellets with hot methanol, and the absorption spectrum was determined with a Beckman DB spectrophotometer (Fig. 9). The single broad peak near 405 nm resembles the Soret band of a porphyrin. The porphyrins and their derivatives are known to be potent photosensitizers in other systems and possibly this is the case in _Acanthamoeba_.

**DISCUSSION**

Other authors have reported the photolability of complex organic media (11, 12, 17). Phelps (17) found that riboflavin was the component of Proteose peptone which was destroyed by light and that its addition to irradiated media restored the growth rate to normal in _Tetrahymena_ cultures. Supplementing preirradiated media with

FIG. 8. Effect of an 8-hr light pulse on a population of amoebae in exponential growth. Curve A, control culture continuously in dark; curve B, experimental culture kept in dark except for a light pulse at intensity of $8.7 \times 10^4$ ergs per sec per cm$^2$.

FIG. 9. Absorption spectrum of a pigment extracted from _Acanthamoeba_ with hot methanol. Cells were washed three times with 0.15 M KCl before extracting to remove OGM.
riboflavin, however, did not increase the growth rate of the amoebae. Lee (11) found that thiamine and flavine mononucleotide (FMN) were destroyed in YE exposed to light. Acanthamoeba has no requirement for FMN but does for thiamine. The addition of thiamine after preirradiation of OGM did not restore the growth rate to that of the control. The identity of the photolabile material(s) was not pursued further and remains conjectural.

Regardless of the nature of these material(s), the important point is whether the photochemical change in the OGM accounts for the decreased growth rate observed in continuously irradiated cultures. The light pulse studies clearly indicate that light directly affects the amoebae and that the growth inhibition attributable to changes in the OGM represents only a small fraction of the total inhibition observed. The factor determining the rate of exponential growth in continuously irradiated cultures is the interaction of light with amoebae and not OGM.

Of the five original hypotheses of how light causes growth inhibition, two cannot be separated on the basis of the data here. Light could be acting directly on a rate-limiting cellular process(es) via an endogenous or exogenous photosensitizing agent. The latter is unlikely because growth inhibition has been observed in some preliminary experiments with Acanthamoeba by using an entirely different medium, Adam's DM2 (1), and a different method of culturing, a chemostat. Briefly, the results of this experiment were as follows: when the amoebae were exposed to cool white fluorescent light at 500 ft-c, the previously steady chemostat population level started to decline and kept doing so until the light was turned off. The cell population began to increase toward the normal level during the dark period but the experiment was terminated for other reasons before the original level was reached. This experiment simply shows that growth inhibition is independent of the type of medium used and implies that the amoebae are photosensitive.

The discovery of a pigment in Acanthamoeba supports the idea that light is directly affecting the cells though it does not preclude the action of exogenous photosensitizers. The identity of this pigment will have to be confirmed and its participation in the observed photo-inhibition demonstrated before an unequivocal statement about the photosensitivity of the amoebae can be made.

ACKNOWLEDGMENTS

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LITERATURE CITED