Ultraviolet survival curves were obtained for four species of marine phytoplankton algae. All but *Cylindrotheca fusiformis* exhibited adherence to strict first-order kinetics. The "shouldered" survival curve of *C. fusiformis* suggests the presence of a repair mechanism or a multiplicity of targets in this organism. The survival curves indicated an order of sensitivity in which *Ditylum brightwelli* was least sensitive to the lethal action of 2537 Å radiation and *Rhodomonas* sp. most sensitive. Cell size alone could explain this order of sensitivity. No significant correlation was observed between pigment content and ultraviolet survival among the four species when grown under similar nutrient conditions. However, *Cylindrotheca fusiformis* was more highly pigmented when cultured using NO$_3^-$ as the nitrogen source than when grown on NH$_4^+$. The more highly pigmented type was found to be less sensitive to the lethal action
of 2537 Å radiation. The effects of ultraviolet radiation on growth rates were examined and found to be depressive for *C. fusiformis* grown on NO₃⁻, stimulative for the same organism grown on NH₄⁺, depressive for *Rhodomonas* sp., and ineffective on the growth rates of *Amphidinium carterii* and *Ditylum brightwelli*. 
The Ultraviolet Photobiology of Marine Phytoplankton

by

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THE ULTRAVIOLET PHOTOBIOLOGY OF MARINE PHYTOPLANKTON

INTRODUCTION

The natural environment of marine phytoplankton is virtually devoid of radiation of lethal ultraviolet (UV) wavelengths. This condition renders the survival value of ultraviolet repair systems minimal among earth's microorganisms. More important, it points to the possibility of studying the effects of ultraviolet radiation on biological systems without the complications which arise from the complex array of repair systems exhibited by some of the more popular experimental organisms. To demonstrate the simplicity of the kinetics of ultraviolet damage in several species of marine phytoplankton algae is one of the objectives of the work presented here. Other objectives include comparison of the relative lethality of 2537 Å radiation among these organisms, with an attempt to explain this relative sensitivity in terms of cell size and pigment content. In addition, the effects of ultraviolet radiation on growth rates are examined.

The solar constant has an average value of 2.0 cal/cm²/min or $1.396 \times 10^6$ ergs/cm²/sec. About half of this energy is concentrated in the visible portion of the electromagnetic spectrum, about 40 percent in the infrared, and about ten percent in the ultraviolet.
Only about 0.1 percent of the energy is at wavelengths shorter than 2000 Å (Koller, 1965).

The short-wavelength limit of the solar radiation reaching the surface of the earth is approximately 2900 Å, depending in small part on the latitude and altitude under consideration (Robinson, 1966). The absence of wavelengths shorter than this is due primarily to absorption by a layer of ozone concentrated in the stratosphere. This ozone is formed from oxygen in the atmosphere by a photochemical reaction, involving dissociation of an oxygen molecule by a quantum of wavelength less than 2424 Å (Koller, 1965). Ozone itself has a strong absorption band that extends from about 2200 Å to 3200 Å, with a maximum at about 2530 Å. The average amount of ozone in the atmosphere is equivalent to about 3.0 mm at standard temperature and pressure, the absolute amount depending upon latitude and season. The range falls between 1.0 and 4.0 mm (Coblentz and Stair, 1935).

Absorption of ultraviolet radiation by air in the lower atmosphere is slight in comparison to that by ozone. While the transmission of one meter of air is only ten percent at 2050 Å, it is 99 percent at 2200 Å (Koller, 1965). However, the presence of pollutants can greatly alter the extinction of ultraviolet in the lower atmosphere.

The intensity of the ultraviolet radiation reaching the surface
of the earth is largely a function of air mass or, more properly, the amount of ozone. Thus it would be expected to vary with time of day, season, latitude and altitude. The variation with time of day was shown by Coblentz and Stair (1936), who showed that the intensity of wavelengths less than 3132 Å was highest when the altitude of the sun was at maximum. These investigators (Coblentz and Stair, 1944) also found that on the clearest days in Washington, D.C. (latitude 39°N), the ultraviolet radiation of wavelengths 3132 Å and shorter, incident on a horizontal plane from the sun and the whole sky at midday, ranged from about 180 μW/cm² (18 ergs/mm²/sec) in midsummer to about 30 μW/cm² (3 ergs/mm²/sec) in midwinter.

In a study of the variation in ultraviolet intensity with altitude (Coblentz and Stair, 1935), they observed an increase of 40 to 50 percent in the intensity of the band of wavelengths between 2900 Å and 3130 Å in rising two kilometers (7,000 ft) above sea level. In this study it was also noted that the intensity of short-wavelength ultraviolet was greater at a sea-level station in the tropics than at a similar station at a higher latitude. Coblentz, Gracely and Stair (1942) found that, for the same solar altitudes, the intensity of ultraviolet at 78°N was somewhat lower than at 39°N.

The penetration of ultraviolet radiation in natural waters is highly dependent upon turbidity, the types and amounts of suspended materials. Lenoble (1955) measured the attenuation coefficients for
UV in sea water in the vicinity of Monaco and obtained values very similar to those for distilled water. She resolved the total attenuation coefficient into two terms, one for absorption and another for scattering, and found that the scattering term was nearly twice the absorption term at the shorter wavelengths (3000 to 3500 Å), while at the longer wavelengths the two were nearly equal.

During the course of the past one hundred years, the response of virtually all types of living organisms to ultraviolet radiation has come under the scrutiny of one investigator or another, sometimes with surprising results. In general, most of the effects of UV on living systems are deleterious, and the extent of these effects is dependent upon a variety of factors, including the size of the organism, the degree and composition of pigmentation, and the presence or absence of repair systems.

In recent years, research in ultraviolet photobiology has focused on microbial systems; i.e. viruses, bacteria, fungi, protozoa and, to a lesser extent, algae. Furthermore, in the continuing search for an elucidation of the mechanism of mutation, the inactivation of transforming principle and the photochemistry of nucleic acids and proteins have received tremendous impetus. Marine phytoplankton algae have been generally ignored in this respect. However, it has been suggested (Halldal, 1967) that phytoplankton, both freshwater and marine, are more sensitive to near ultraviolet
(3100-3900 Å) than other organisms. This may prove to be the case also for far ultraviolet (1900-3100 Å). Rupert (1964) has reasoned that studies employing different wavelengths in the 2000-3000 Å region indicate generally that, while wavelengths approaching 2000 Å may have effects different from the longer ones, those near 3000 Å are similar in many ways to 2537 Å, the emission line of mercury used in most studies of ultraviolet action.

In a study of the action of ultraviolet radiations on Spirogyra, Gibbs (1926) demonstrated the lethality of wavelengths 2378 Å to 3126 Å for S. submaxima affinis and S. nitida affinis. Meier (1932, 1934) found similarly that wavelengths shorter than 3022 Å were lethal to Chlorella vulgaris, while irradiations with wavelengths longer than 3022 Å were without effect. She further found (Meier, 1936) that the assumption of time and dose rate reciprocity held to a good approximation for this organism; i.e. percent lethality was proportional to total dose only regardless of dose rate. Swann and del Rosario (1932) had obtained essentially the same results with Euglena. Nybom (1953) has shown that only a negligible fraction of Chlamydomonas eugametos killed by 2537 Å radiation undergo cell division before death.

Redford and Myers (1951), in a study of the effects of UV (2537 Å) on the metabolism of Chlorella pyrenoidosa, obtained a straight line when the logarithm of the surviving fraction was plotted
against dose. Although Sasa (1961) expressed his data differently, when plotted on a semilogarithmic scale, they yield the same result for *Chlorella ellipsoidea*. On the other hand, Van Baalen (1968) obtained a "shouldered" survival curve for *Agmenellum quadruplicatum*, a coccoid blue-green alga which exhibits photoreactivation (the reversal of UV damage by light of another wavelength). Such a departure from first-order kinetics is also manifest by the data of Nybom (1953) for *Chlamydomonas eugametos*, which also exhibits photoreactivation. A similar curve was obtained by Hill, Schiff and Epstein (1966a, 1966b) for viability (colony-forming ability) of *Euglena gracilis*, an inactivation which is photoreactivable to the extent of about 60 percent. Another photoreversible UV inactivation which has also been found to exhibit a "shouldered" inactivation curve is green colony formation in *Euglena gracilis* (Lyman, Epstein and Schiff, 1961; Schiff, Lyman and Epstein, 1961; Cook, 1963; Cook and Hunt, 1965; and Petropoulos, 1964). In contrast to the case for colony formation, the inactivation of green colony formation by ultraviolet radiation in *Euglena gracilis* is photoreversible with efficiencies approaching 100 percent (Schiff *et al.*, 1961; Hill *et al.*, 1966b).

For a study of the relative sensitivity of several species of algae to ultraviolet radiation of 2537 Å, McLeod and McLachlan (1959) used the freshwater green algae *Chlorella vulgaris*,
Scenedesmus quadricauda and Ankistrodesmus falcatus, the freshwater yellow-green alga, Botryococcus braunii, the marine green algae, Chlorella sp., Platymonas subcordiformis and Dunaliella euchlora, and the marine diatoms, Phaeodactylum tricornutum and Skeletonema costatum. They reported that the diatoms were most sensitive, while Scenedesmus quadricauda and the marine green algae were more sensitive than the other freshwater species.

A stimulative effect of ultraviolet radiation on the growth of Stichococcus bacillaris has been reported (Meier, 1939; Meier Chase, 1941). These findings are in contrast to those of Haildal (1961) and Sasa (1961) for Platymonas subcordiformis and Chlorella ellipsoidea, respectively. Kumar (1963) exposed the unicellular blue-green alga Anacystis nidulans to 2537 Å radiation to obtain an ultraviolet-resistant strain which was characterized by a lower carotenoid:chlorophyll ratio as well as a slower growth rate.

Clayton, Bryan and Frederick (1958) subjected wild-type and carotenoidless Rhodospirillum spheroides, a purple sulfur bacterium, to doses of ultraviolet radiation and suggested that carotenoids protect the cell from UV damage. Kunisawa and Stanier (1958), however, were unable to show a difference in ultraviolet sensitivity using wild-type and carotenoidless strains of Corynebacterium poinsettiae. In a study of the ultraviolet inactivation of green colony formation of several different types of Euglena gracilis, Hill et al.
(1966a) observed that in darkgrown cells and in an X-ray-induced mutant, the dose required to produce a single inactivation event was proportional to the chlorophyll content of the cells. However, in hyperdeveloped cells which contained abnormally high amounts of chlorophyll, the correlation did not hold.

The principal objectives of the work presented here are (1) the comparison of the ultraviolet sensitivities of several species of marine algae through the analysis of survival curves, (2) tests of the correlations of sensitivity with cell size and pigment content, and (3) the evaluation of the effects on growth rate. Strickland et al. (1969) have noted that phytoplankton algae are more or less pigment-ed, depending on the nitrogen source utilized during growth. This situation is examined here with respect to the importance of pig-

ments in determining ultraviolet sensitivity.
MATERIALS AND METHODS

Outline of the Method

When cultures of the experimental organisms reached logarithmic growth phase, aliquots were pipetted into petri dishes and irradiated for various lengths of time. Sterile medium was then added and cell counts were made periodically during the course of the next several days. The cell counts for a given sample were then subjected to regression analysis to obtain viable cell number and growth rate. Pigment analyses were performed on the remaining volume of cell suspension to obtain preirradiation contents of chlorophyll a and carotenoids. Cell volumes were estimated from linear dimensions.

The Organisms

The organisms used in these experiments were two unicellular marine diatoms, Cylindrotheca fusiformis and Ditylum brightwelli, a marine dinoflagellate, Amphidinium carterii, and a marine cryptomonad, Rhodomonas sp. Unicellular organisms were used exclusively in order to avoid the theoretical complications produced by shading in multicellular aggregates. Other factors influencing the choice of experimental organisms were ease of culturing, ease of counting, cell size and pigmentation.
Media and Growth

For all the organisms except *Cylindrotheca fusiformis*, the growth medium used was Millipore-filtered (0.45 μ porosity) sea water, enriched according to the formula of Ryther and Guillard (1959), except that thiourea was substituted for sodium thiosulfate and 0.2 g of sodium bicarbonate per liter of medium was added as a stock solution to replace that lost during autoclaving. *Cylindrotheca fusiformis* was cultured as described above except that artificial sea water, prepared as prescribed by Lyman and Fleming (1940), was substituted for Millipore-filtered sea water. For the study involving different nitrogen sources, NH₄Cl was substituted for NaNO₃.

Batch cultures and irradiated samples were grown under 200-300 ft-candle illumination at a temperature of 21-23 C.

Irradiation and Counting

When batch cultures reached logarithmic growth phase as determined by visual cell counts, 10-ml samples in petri dishes were irradiated under a G. E. G15T8 germicidal lamp; a low-pressure mercury vapor lamp which characteristically emits about 90 percent of its energy output at the 2537 Å resonance line of mercury. The dishes were positioned directly beneath the lamp on a shelf at a distance of 16 cm from the lamp. Only the central 12 cm of the
lamp were utilized. Under these conditions, samples were exposed to a dose rate of $35 \text{ ergs/mm}^2/\text{sec}$ as determined with a YSI-Kettering Model 65 Radiometer.

After irradiation 10-ml aliquots of sterile medium were added to all irradiated samples and controls, which were then replaced under culture conditions. Visual cell counts were made periodically during the course of the following week, using a Fuchs-Rosenthal counting chamber for all species except *Ditylum brightwellii*, which was counted with the aid of a Sedgwick-Rafter counting chamber. *Amphidinium carterii* and *Rhodomonas* sp. were fixed with Lugol's iodine prior to counting.

### Viable Cell Counts and Growth Rates

In the great majority of ultraviolet survival studies, the organisms of choice have been and continue to be those which are readily cultured on solid media. The criterion for cell viability then is the ability to reproduce and form a visible colony within a reasonable length of time. Survival ratios are obtained simply by comparing the number of colonies produced by an irradiated suspension of cells with the number produced by a nonirradiated control suspension. A disadvantage of this method is that it gives no quantitative information regarding the growth rates involved. However, when working with photosynthetic organisms which may be cultured
heterotrophically, it possesses the advantage of enabling the investigator to separate damage to chloroplasts from damage to nuclear and mitochondrial systems. This is made possible by the fact that, at sublethal doses of ultraviolet radiation, inactivation of chloroplast development gives rise to "bleached" or fractionated colonies.

The alternative method employed here is that of Redford and Myers (1951). It assumes that the growth rate is constant for some time after irradiation and that the postirradiation number of viable cells may be obtained from periodic cell counts by plotting the logarithm of the cell numbers against postirradiation time, followed by linear extrapolation to time of irradiation. In other words, it is assumed that growth follows the equation

\[ N_t = N_0 e^{kt} \]  

(1)

or

\[ \log N_t = \log N_0 + k't \]

and

\[ \frac{d \log N_t}{dt} = k' \]

where \( N_t \) is the cell number at time \( t \), \( N_0 \) the original cell number, \( k \) the growth constant, and \( k' = k/2.3 \), a conversion necessary if cell numbers are to be expressed in log_{10} units. Thus it can be
seen that, when log \( N_t \) is plotted against time, the y-intercept will be \( N_0 \) and the slope \( k' \).

It should be noted that the above equations describe exponential growth and do not account for a lag phase. However, Spencer (1954), in a study of the growth of *Phaeodactylum tricornutum*, found that when exponentially growing cells were used as an inoculum, no lag in growth could be detected. This was found to be the case in the present study also.

When a suspension of cells is subjected to a dose of lethal radiation, an apparent lag phase might be expected as a result of the inclusion of nonviable cells in early cell counts. This situation could be formulated as

\[
N = N_v e^{kt} + N_0 (1 - e^{-RD})
\]  

(2)

where \( N \) is the number of cells counted at a given time \( t \), \( N_v \) the number of viable cells, \( R \) the rate of change of viable cell number with dose, and \( D \) the magnitude of the dose. But, as \( t \) increases, the second term on the right-hand side of the equation becomes small with respect to the first and the observed growth rate approaches the true growth rate asymptotically. Therefore,

\[
\log N \approx \log N_v + k't
\]  

(3)

and

\[
\frac{d \log N}{dt} = k'.
\]
The error resulting from the counting of nonviable cells is further reduced by autolytic processes and rupture induced by agitation before withdrawing samples for counting. It can thus be seen that postirradiation cell counts may be used to estimate $N_v$ and $k'$.

A method of refining the estimates of $N_v$ and $k'$ suggested itself, and that was one of successive approximations. It might be expected that, if the estimate of $N_v$ obtained in the first approximation were used to revise the original cell counts and regression analysis performed on the resultant data, this would result in a more accurate estimate. This process could be repeated a number of times until the change in $N_v$ from one approximation to the next was negligible.

However, when the data for survival of *Amphidinium carterii* were subjected to this treatment, it resulted in a lowering of the correlation coefficient and an elevation of the standard deviation of $N_v$ in all cases but one. The conclusion reached here was that this method, instead of straightening the line obtained in the first approximation, actually resulted in a deviation from linearity.

**Pigment Analysis**

Analyses for chlorophyll a and carotenoids were performed on the same batch culture from which samples had been taken for irradiation. The method employed was that of Strickland and Parsons (1965) and the instrument an Hitachi Perkin-Elmer Model 111.
Spectrophotometer.

In order to express pigment concentrations relative to cell volume, cell volumes were estimated from linear dimensions with the aid of an ocular grid. It was assumed that all the cells were approximately cylindrical.
RESULTS AND DISCUSSION

Growth Curves

Of the growth curves used to define survival, all exhibited correlation coefficients above 0.90, while 80 percent showed correlation coefficients greater than 0.98. An average of six cell counts was used to define a growth curve. It would thus appear that, for the purposes of the work described here, the assumption of logarithmic growth according to equation 1 (p. 12) is reliable.

Survival

Survival curves for the four species of marine phytoplankton are shown in Figures 1-3. The assumption of strict linearity holds to a close approximation for all species except Cylindrotheca fusiformis; i.e. ultraviolet survival can be described by equation 2 (p. 13). The data presented here, when fitted to the log-linear regression model, yielded correlation coefficients of 0.90 to 0.96.

The "shouldered" survival curve exhibited by C. fusiformis has two, perhaps three, possible explanations. The first is that postulated by Harm (1968) to explain the shape of the survival curve of Escherichia coli. He suggested that, at low doses and dose rates, a repair mechanism (probably photoreactivation) operates at a rate
Figure 1. Survival of *Rhodomonas* sp. and *Amphidinium carterii*.
Figure 2. Survival of Ditylum brightwelli.
Figure 3. Survival of Cylindrotheca fusiformis grown on NO$_3^-$ and grown on NH$_4^+$. 

DOSE (ergs/mm$^2$)

N/N$_o$ x 100

DOS (sec)
sufficient to excise all lesions. However, at higher doses and dose rates, the rate of production of UV lesions becomes greater than the rate at which they can be repaired. The second explanation is that of Hill et al. (1966b); i.e. a multiplicity of targets is responsible for the deviation from strict first-order kinetics. A possible third hypothesis might be that an enzyme operating in a repair system might itself be inactivated at high doses of ultraviolet radiation.

The plots of survival vs. dose exhibit an order of sensitivity in which *Ditylum brightwelli* is least sensitive and *Rhodomonas* sp. most sensitive of the organisms grown on similar media. F-tests for common regression revealed a significant difference between the survival curves for any two species grown under similar nutrient conditions (confidence level of 90 percent), but failed to show a significant difference between the lines for *Rhodomonas* sp. and *Cylindrotheca fusiformis* grown on NH\(_4^+\). The lines describing survival of *C. fusiformis* cultured in media containing the two different nitrogen sources, NO\(_3^-\) and NH\(_4^+\), were found to be significantly different at all standard confidence levels.

**The Influence of Cell Size**

The relationship between cell volume and ultraviolet sensitivity is illustrated in Figure 4. The regression of D\(_{50}\), the dose for 50 percent survival, with the logarithm of cell volume is linear with a
Figure 4. Dose for 50 percent survival ($D_{50}$, sec) vs. cell size.
correlation coefficient of 0.87. Therefore, cell size alone is sufficient to account for the order of sensitivities observed above.

**Pigments**

Multiple regression analysis and tests for significance of regression coefficients could establish no significant correlation between ultraviolet sensitivity ($D_{50}$) and chlorophyll a content, carotenoid content or carotenoid:chlorophyll ratio. It would thus appear that, if pigments exert any influence at all on ultraviolet sensitivity in these organisms, it is slight in comparison to the influence of cell size. As noted previously, algae grown with NO$_3^-$ as a nitrogen source are more highly pigmented than those grown with NH$_4^+$. This is borne out by the pigment data (Table 1) for *Cylindrotheca fusiformis*, those grown on NO$_3^-$ having developed nearly twice the pigment content of those grown on NH$_4^+$. The fact that the more highly pigmented form is less sensitive to 2537 Å radiation suggests a possible protective function of pigments against UV damage (Figure 3). However, as Strickland _et al._ (1969) have shown, changes in nitrogen sources can alter many phases of algal metabolism.

**Effects on Growth Rates**

The growth rates (in log$_{10}$ units) are listed in Table 2 for irradiated samples and nonirradiated controls. Using a Student's
Table 1. Cell volumes, chlorophyll content and carotenoid content of four species of marine phytoplankton algae.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell Volume ($\mu m^3$)</th>
<th>Chlorophyll a (mg/$\mu m^3$)</th>
<th>Carotenoids (m-SPU/$\mu m^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodomonas sp.</td>
<td>$2.71 \times 10^1$</td>
<td>$8.52 \times 10^{-10}$</td>
<td>$11.30 \times 10^{-10}$</td>
</tr>
<tr>
<td>D. brightwelli</td>
<td>$6.54 \times 10^4$</td>
<td>$3.84 \times 10^{-10}$</td>
<td>$3.95 \times 10^{-10}$</td>
</tr>
<tr>
<td>C. fusiformis (NO$_3^-$)</td>
<td>$1.07 \times 10^2$</td>
<td>$5.70 \times 10^{-10}$</td>
<td>$4.30 \times 10^{-10}$</td>
</tr>
<tr>
<td>C. fusiformis (NH$_4^+$)</td>
<td>$1.07 \times 10^2$</td>
<td>$2.70 \times 10^{-10}$</td>
<td>$2.50 \times 10^{-10}$</td>
</tr>
<tr>
<td>A. carterii</td>
<td>$6.11 \times 10^2$</td>
<td>$2.83 \times 10^{-10}$</td>
<td>$8.96 \times 10^{-10}$</td>
</tr>
</tbody>
</table>
Table 2. Growth rates ($k' \times 10^3$) of four species of marine phytoplankton after irradiation with 2537 Å.

<table>
<thead>
<tr>
<th>Dose (sec)</th>
<th>0</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fusiformis ($\text{NO}_3^-$)</td>
<td>23.6</td>
<td>21.9</td>
<td>-</td>
<td>21.5</td>
<td>21.8</td>
<td>-</td>
<td>21.7</td>
<td>-</td>
<td>20.2</td>
<td>19.5</td>
<td>15.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. fusiformis ($\text{NH}_4^+$)</td>
<td>15.2</td>
<td>-</td>
<td>17.1</td>
<td>-</td>
<td>22.4</td>
<td>-</td>
<td>-</td>
<td>32.7</td>
<td>-</td>
<td>26.8</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. carterii</td>
<td>9.6</td>
<td>8.3</td>
<td>-</td>
<td>10.1</td>
<td>8.0</td>
<td>-</td>
<td>8.4</td>
<td>-</td>
<td>8.4</td>
<td>8.1</td>
<td>8.4</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Rhodomonas sp.</td>
<td>21.3</td>
<td>22.1</td>
<td>19.9</td>
<td>17.2</td>
<td>11.6</td>
<td>11.4</td>
<td>11.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D. brightwelli</td>
<td>8.0</td>
<td>-</td>
<td>7.6</td>
<td>-</td>
<td>4.5</td>
<td>-</td>
<td>-</td>
<td>7.4</td>
<td>-</td>
<td>9.6</td>
<td>-</td>
<td>-</td>
<td>7.8</td>
</tr>
</tbody>
</table>
t test statistic for differences of slope (90 percent confidence level), it was found that the treatment significantly depressed the growth rate of *Cylindrotheca fusiformis* grown on NO$_3^-$ at the two highest doses, while this organism exhibited significant growth-rate stimulation at the two highest doses when grown on NH$_4^+$. *Amphidinium carterii* showed no significant effect of ultraviolet irradiation on growth rate. The growth rate of *Rhodomonas* sp. was significantly depressed at the three highest doses, while the growth rate of *Ditylum brightwellii* was virtually unaffected. Low doses of 2537 Å radiation evidently have stimulative effects on some organisms and depressive effects on others, although the reasons for this disparity are not readily apparent. Although the case of *Cylindrotheca fusiformis* grown on media containing different nitrogen sources is particularly puzzling, an hypothesis might be offered. The most obvious difference between the nitrate- and ammonia-grown organisms is the presence of and dependence on nitrate reductase in the former. The possible inactivation of this nitrate-reducing system by ultraviolet radiation might account for a depressed growth rate. The subsequent limitation placed on the nitrate reductase activity would result in depressed rates of protein synthesis as well as nucleic acid and chlorophyll synthesis. The inactivation of the nitrate reductase system might be direct; i.e. inactivation of the enzyme, or indirect; e.g. inactivation of the adenosine triphosphate (ATP)
necessary to drive the system. In this regard, Strickland et al. (1969) noted an increase in ATP synthesis when *Ditylum brightwellii* shifted from ammonia to nitrate metabolism. Growth rates might be enhanced by low doses of ultraviolet radiation as a response to lowered cell density and subsequent increase in nutrient availability. At higher doses this response would be negated by increased damage to enzyme systems.

**Ecological Significance**

While studies of the wavelength dependence (action spectra) of ultraviolet damage to cells (Lyman et al., 1961; Giese, 1964; and Koller, 1965) have demonstrated that the solar radiation reaching the surface of the earth does contain wavelengths which are lethal to microorganisms, the ecological implications for marine phytoplankton are slight. The biological effectiveness of 3000 Å radiation is on the order of three percent that of 2537 Å. Assuming that 100 ergs/mm$^2$ might represent the maximum dose that a phytoplankton cell might be exposed to in nature, this would be roughly equivalent to 3 ergs/mm$^2$ of 2537 Å radiation. Substituting this value for the dose and the value for the slope of the survival curve for *Rhodomonas* sp. (Figure 3, $R = -0.0065$) into the expression describing UV death (equation 2, p. 13), the lethality would be 0.3 percent. Since *Rhodomonas* sp. was the most sensitive organism examined
in this respect, this value could be considered a maximum for marine phytoplankton. However, in order to receive a dose of 3000 Å radiation as high as 100 ergs/mm², these organisms would have to remain located at the surface of the ocean for an entire day. Such a circumstance would be improbable at best.

It is interesting to speculate concerning the implications of ultraviolet photobiology for possible life on other planets in our solar system. The situation on Mars would be in great contrast to that on earth. Confronted by a rarefied atmosphere deficient of oxygen, microorganisms would not have the benefit of a protecting shield of ozone and would be forced to cope with a new, potentially lethal element in the radiation environment. Proteins and nucleic acids (the loci of UV damage to cells) could not function as they do in terrestrial microorganisms unless a highly efficient repair mechanism were to evolve concomitantly. Likewise, the success of any microorganisms which might be carried to Mars by an earth-based spaceship would be tenuous at best.

The close correspondence of the absorption spectrum of nucleic acid, the absorption spectrum of ozone and the action spectrum of ultraviolet damage (Giese, 1964), all with wavelength maxima at about 2600 Å, has yet to be explained in the context of the evolution of life on earth. This coincidence is consistent with the theory that life on earth arose from a series of condensations of organic
free radicals produced from atmospheric gases by ultraviolet radiation. It is reasonable that the macromolecular products of these reactions would themselves be unstable to ultraviolet radiation. It is interesting to note that the series of reactions which gave rise to the first chemoheterotrophic organisms might have continued infinitely had it not been brought to a halt with the rise of photosynthetic autotrophs and the ozone layer. The primeval ocean might have offered the first organisms some respite from the damaging action of ultraviolet radiation.

Possible Further Research

The "shouldered" survival curve of Cylindrotheca fusiformis (Figure 3) suggests a possible repair mechanism operating in this organism. An investigation was undertaken to ascertain whether or not ultraviolet damage was actually photoreversible in C. fusiformis. Technical difficulties complicated the study insofar as this species could not be cultured in the dark. Since that time, a possible technique has suggested itself. Action spectra of photoreactivation (Rupert, 1964) in many organisms have shown that this phenomenon is restricted to the lower portion of the visible wavelength range (3000-5000 Å). It should be feasible then, with suitable optical filters, to culture obligate phototrophs under light which permits photosynthesis, while being deficient of photoreactivating wavelengths.
Survival curves for four species of marine phytoplankton algae have been described. It would be interesting to compare the sensitivities of these organisms with those of freshwater phytoplankton algae, terrestrial algae and the cryophilic snow algae indigenous to high altitudes where ultraviolet radiation is more intense than at sea level.

A stimulative effect of ultraviolet radiation on the growth rate of *Cylindrotheca fusiformis* grown on NH$_4^+$ and a depressive effect for the same organism grown on NO$_3^-$ have been observed. The reasons for this variation in the effect of UV on growth rate require further investigation.

A relationship between pigment content and ultraviolet survival has been observed in *C. fusiformis*, although the relationship may be indirect and reflect differences in physiology between algae grown on different nitrogen sources. It should be possible, however, by varying light and temperature regimes, to vary pigment content. In this manner, the correlation between pigment content and survival might be tested.
CONCLUSIONS

1. The logarithmic extrapolation method was found to be satisfactory for estimating survival and growth rates of obligate phototrophs in ultraviolet studies.

2. Survival curves for four species of marine phytoplankton algae grown under similar nutrient conditions were found to exhibit an order of sensitivity which can be accounted for by cell size alone.

3. *Cylindrotheca fusiformis* grown on different nitrogen sources showed differences in their survival curves which may reflect differences in pigment content. The more highly pigmented form was less sensitive to the lethal action of UV, suggesting a possible protective function of pigments.

4. No correlation could be observed between pigment content and ultraviolet survival among the four species grown under similar nutrient conditions.

5. *Cylindrotheca fusiformis* exhibited a "shouldered" survival curve, indicating either a repair mechanism or a multiplicity of targets.

6. Ultraviolet irradiation can result in depressed or stimulated growth rates. *Cylindrotheca fusiformis* exhibits both responses, depending on the nitrogen source utilized during growth.
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