Action Spectra for Two Effects of Light on Luminescence in *Gonyaulax polyedra*

B. M. SWEENEY, F. T. HAXO, and J. W. HASTINGS

ABSTRACT The luminescence of the marine dinoflagellate *Gonyaulax polyedra* shows an endogenous diurnal rhythm. The effect of light during the phase of low luminescence capacity may be observed as an enhancement of luminescence during the subsequent bright phase. During the bright phase, however, illumination diminishes the capacity for luminescence. The action spectra for these two effects of light have been determined, and the major pigments of *Gonyaulax* have been examined. A consideration of the action spectrum and the pigment complement of *Gonyaulax* suggests that photosynthesis during the day is responsible, directly or indirectly, for the enhancement of luminescence during the following night. Photoinhibition of luminescence is in part attributable to light absorbed by the photosynthetic pigments. However, activity observed in the far red region of the spectrum beyond the absorption maximum of chlorophyll *a* suggests that an additional pigment, present in small amounts, may also act as sensitizer for photoinhibition.

INTRODUCTION

The photosynthetic marine dinoflagellate, *Gonyaulax polyedra*, is one of many organisms which contribute to the "phosphorescence" of the sea. The bioluminescence of laboratory cultures of this dinoflagellate has been the subject of a series of studies by the authors, in which the general features of this process have been described (Haxo and Sweeney (1955); Sweeney and Hastings (1957); Hastings and Sweeney (1958)).

Measurements of the luminescence of cell suspensions under a variety of conditions demonstrated early in the course of these studies that light affects the intensity of luminescence. Two types of light effects have been observed. First, it was apparent that light inhibits luminescence. Second, the intensity of luminescence proved directly dependent on the light intensity during preillumination. In the present study, the action spectra for these two effects

From The Scripps Institution of Oceanography, University of California, La Jolla, and the University of Illinois, Urbana.

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were determined in an effort to identify the sensitizing pigments and to establish the relationships of these effects to other physiological processes.

A number of observations have led to the postulation that luminescence in *Gonyaulax polyedra* is an expression of diurnal endogenous rhythmicity (Sweeney and Hastings (1957); Hastings and Sweeney (1958)). The general features of this intrinsic rhythmicity which must be taken into consideration in determining the effects of light on luminescence capacity are summarized

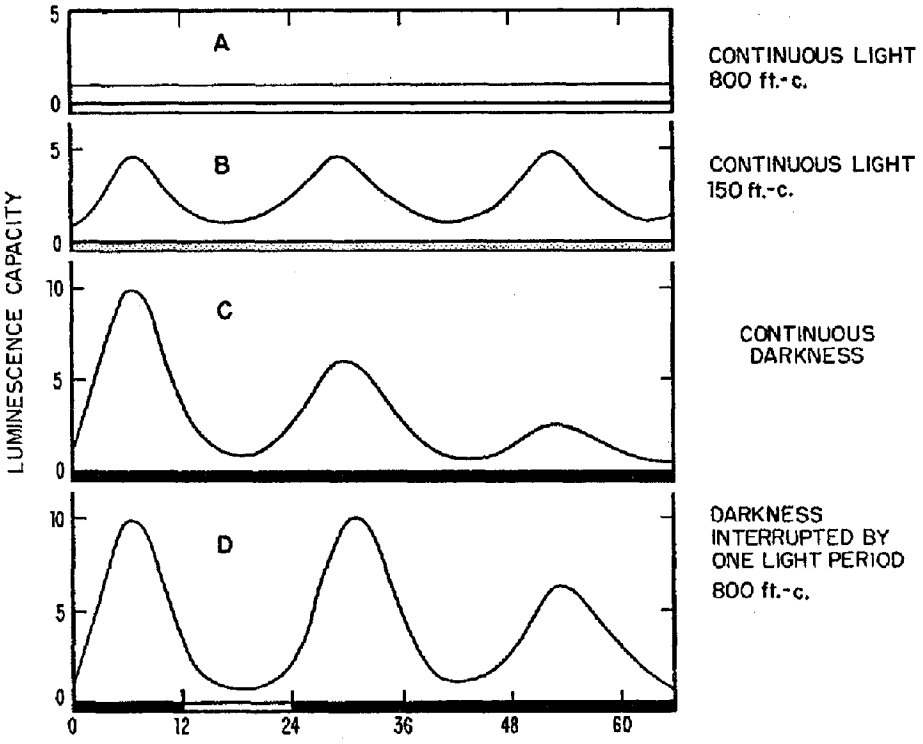

FIGURE 1. Diagrammatic representation of the changes in luminescence capacity of *Gonyaulax polyedra* taken from continuous bright light (800 foot-candles) and maintained at the same temperature (20°C.) in the light and dark regimes indicated by bars along the abscissa. In curve A, the cells remained in bright light; in curve B, the light intensity was reduced to 150 foot-candles at 0 hours; in curve C, the cells were transferred to darkness at 0 hours; and curve D, darkness was interrupted with a single light period (800 foot-candles) between 12 and 24 hours. The difference in the magnitude of the luminescence capacity of the first cycles of curves B and C exemplifies the inhibitory effect of light of intermediate intensities comparable to those employed in determining the action spectrum of photoinhibition. The maximum light inhibition obtainable is represented in curve A. The differences in the magnitude of luminescence capacity at comparable times during the second cycles of curves C and D illustrate the photoenhancement brought about by the preceding light period in curve D.

in Fig. 1. All the curves represent luminescence in cells grown in continuous bright light. When this treatment is continued (Fig. 1, curve A), luminescence remains at a constant low value with no manifestation of rhythmicity. However, on placing such cell suspensions in light of lower intensity (Fig. 1, curve B), the endogenous rhythm of luminescence becomes apparent. On transferring cells to darkness, (Fig. 1, curve C), the luminescence capacity also shows rhythmicity, but the amplitude of the rhythm decreases with each successive cycle. If a light period intervenes between the first and second maximum in luminescence (Fig. 1, curve D), the decrease in amplitude of the following cycle is prevented. The difference in luminescence capacity of the first cycles of curves B and C is a measure of the inhibitory effect of light of medium intensity on the luminescence capacity. Maximum photoinhibition is depicted in curve A. The difference in the magnitude of the luminescence capacity at comparable times during the second cycles of curves C and D illustrates the photoenhancement brought about by the preceeding light period in curve D.

In contrast to the luminous bacteria and fungi which emit light continuously, bright luminescence in *Gonyaulax polyedra* is a response to mechanical stimulation.[1] When cell suspensions are vigorously agitated, luminescence is virtually exhausted during the first minute of stimulation. The term luminescence as used in this study refers in all cases to light emission integrated over the first minute of stimulation.

Methods

MATERIAL For this investigation, unialgal cultures of *Gonyaulax polyedra* were grown in Fernbach flasks at 20°C. with illumination from "cool white" fluorescent lamps at an intensity of about 1000 foot-candles. These lamps were on continuously for 12 hours during each 24 hour period, thus making "day" and "night" 12 hours each. Cultures were inoculated at a cell density of about 300 cells per ml. The volume of each culture was 1500 ml. On the day prior to experimental light treatments, 2 ml. aliquots from a culture were distributed into a number of 12 × 120 mm. pyrex test tubes and maintained in alternating light and darkness until the beginning of the experiment.

The culture medium was composed of 75 per cent filtered sea water, 2 per cent soil extract (Pringsheim (1946)), 2×10^{-3} M KNO_3, 2×10^{-4} M K_2HPO_4, 6×10^{-6} M $FeCl_3$, and 2.7×10^{-5} M ethylenediamine tetraacetic acid (EDTA) at pH 8.0–8.2.

LIGHT SOURCES AND IRRADIATIONS In experiments in which cell suspensions were irradiated with white light, the light source was a bank of cool white fluorescent lamps similar to those used to illuminate cultures during growth. The intensity of white light was measured with a foot-candle meter (Weston model 756) or with an

[1] In addition, a very faint glow may be detected with extremely sensitive instrumentation.

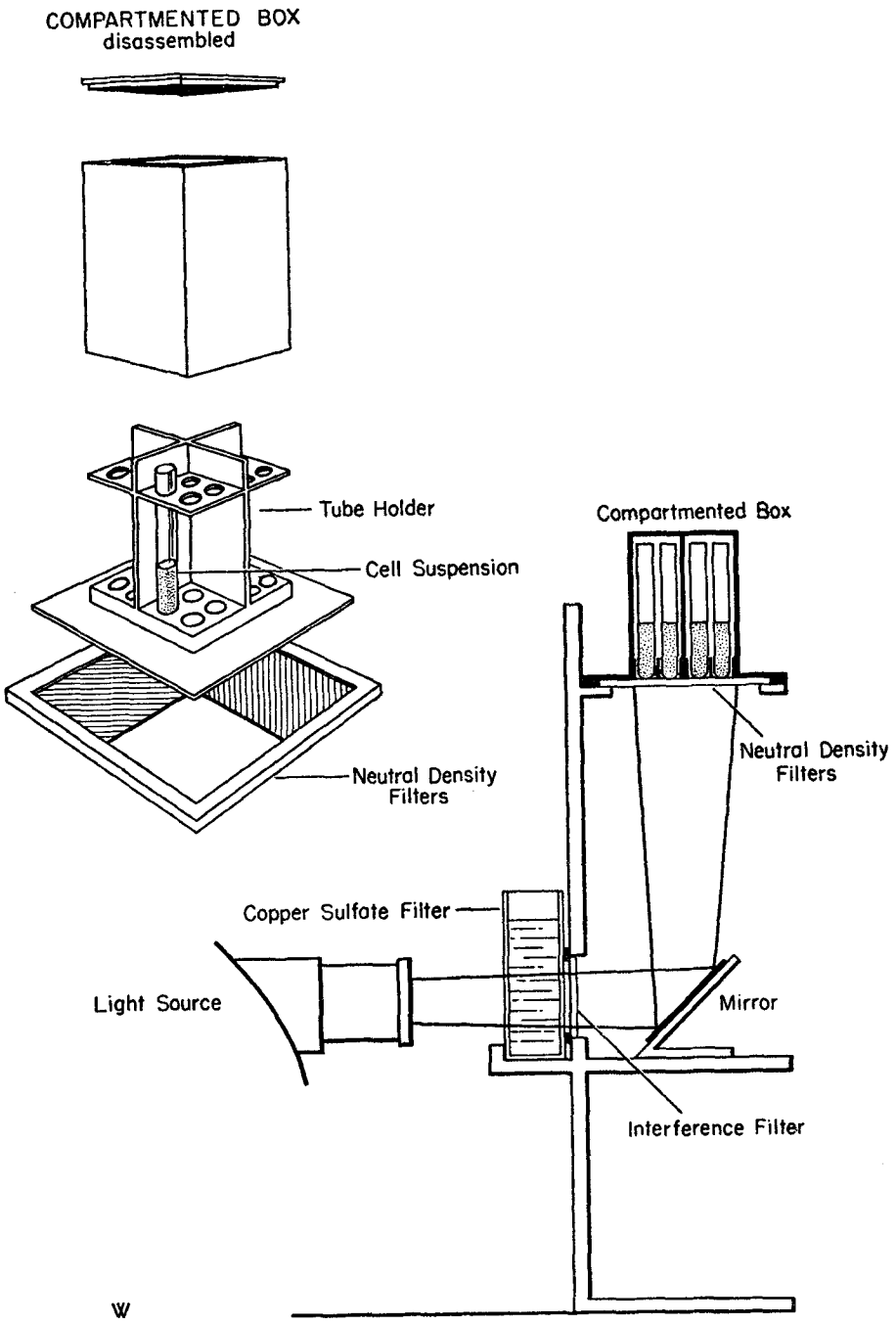

FIGURE 2. Schematic diagram of the optical system employed for the determination of the action spectra of photoenhancement and photoinhibition of luminescence capacity drawn slightly off the median plane to show two compartments and the positioning of four of the sixteen tubes. Box, tube holders, and shielding were of black plastic. Three such units were used with three different interference filters, providing simultaneous irradiation at three wave lengths.

Eppley thermopile. The measured conversion factor for this light source, with a 5 cm. layer of water inserted in the light path, was 46.5 ergs per cm.^{2} per sec. per ft.-candle. Different intensities of white light were obtained either by varying the distance between the lamps and the cell suspension or by interposing in the light path crossed polaroid films.

Monochromatic light was obtained by using interference filters (Balzers). These were found to transmit about 40 per cent of the incident light at the wave length of maximum transmission and to have a half-band pass ranging from 9 to 15 mμ and averaging 11 mμ. Three 1000 watt slide projectors, each with a 5 inch focal length f2.3 lens system, were used as light sources, the intensities being adjustable by a variable transformer. The divergence of the emergent beam was about 7°. As shown in Fig. 2, the optical system included, in sequence, a 5 cm. path of acidified 0.02 M $CuSO_4$,[2] an interference filter, and a front-surfaced mirror set at an angle of 45° to the axis of the light beam. The latter was thus directed onto a compartmented container for simultaneous irradiation at four light intensities, achieved by facing the lower surface of each compartment with different neutral density filters (Wratten). Each compartment accommodated four test tubes.

Light intensities were measured with a photronic cell (Weston model 856, type RR), in conjunction with a microammeter. The photronic cell was masked to the cross-sectional dimensions of a test tube and provided with a collar extending to the position normally occupied by the samples during irradiation. The photronic cell was calibrated in ergs per sec. per cm.^{2} for each interference filter in the optical system described above, using an Eppley thermopile and a standard lamp. All experiments were performed in a constant temperature room at 20 ± 1.5°C.

MEASUREMENTS OF LUMINESCENCE Luminescence was measured with a photomultiplier photometer which integrates the light emitted (Sweeney and Hastings (1957)). The cells were stimulated (Fig. 3) to emit light by bubbling air through the cell suspension for 1 minute, by which time virtually all luminescence has ceased. Recovery subsequent to stimulation is slow and varies under different conditions, hence different aliquots from the same cell suspension were always used to assess luminescence.

PIGMENT STUDIES The fat-soluble pigments were extracted from *Gonyaulax* cells with methanol and separated chromatographically on columns of powdered sucrose, according to the method of Strain *et al.* (1944). From their characteristic absorption spectra in several solvents, chlorophyll *a* and chlorophyll *c* were identified as the only green pigments, and peridinin as the predominant carotenoid. The composition of the *Gonyaulax* carotenoids was not investigated further, although the presence of other components was noted.

The absorption curve of the total fat-soluble pigment was determined in methanol with an aliquot from the original extract. Since attempts to fractionate this extract quantitatively proved unsuccessful, largely due to the difficulty in separating chlorophyll *c* and the polyoxy carotenoids, the contributions of individual pigments or groups of pigments to the total absorption of the extract, as shown in Fig. 6, were

[2] Water was substituted for $CuSO_4$ solution when interference filters transmitting light of wave lengths longer than 705 mμ were used.

estimated as follows. The absorption curves determined for chromatographically separated chlorophyll *a* and chlorophyll *c* in methanol were replotted to provide the best fit of their summated absorptions to that of the total pigment extract in the red region of the spectrum. The absorption of the total carotenoid fraction could then be

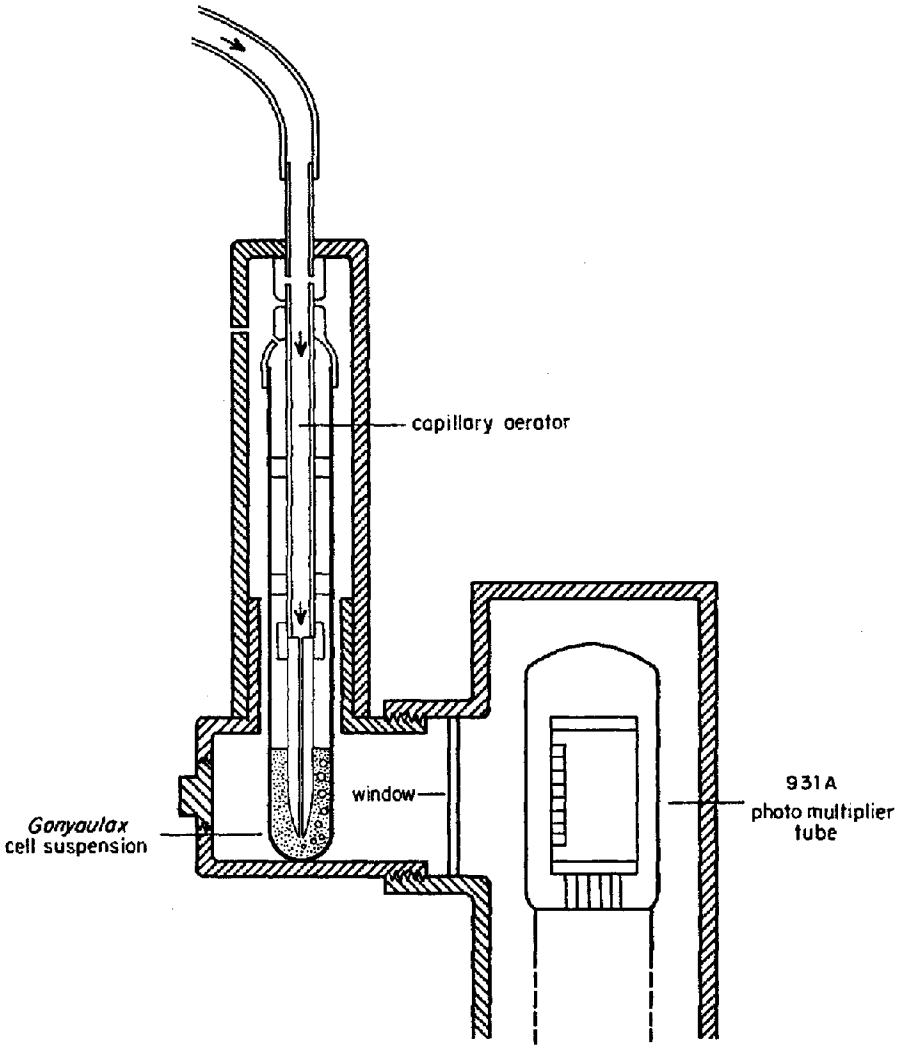

FIGURE 3. Schematic diagram of the apparatus used for stimulating luminescence in *Gonyaulax* by aeration and for detecting the light emitted.

derived as the residual absorption of the total pigment extract not attributable to chlorophylls. Through the quantitative fractionation of the carotenoids from *Gonyaulax*, it was found that all of the absorption of carotenoid fractions at wave lengths greater than 525 mμ was accounted for by peridinin. The contribution of peridinin

to the absorption of the corresponding total extract was therefore estimated by normalizing at this wave length. All absorption curves were determined in detail with a Beckman DU spectrophotometer.

The absorption of intact *Gonyaulax* cells was determined with a concentrated cell suspension, employing the opal glass technique of Shibata, Benson, and Calvin (1954). Settling of the living cells during the measurement was avoided by increasing the viscosity of the suspending nutrient medium by adding a small amount of cellulose gum.[3]

Experimental Results

PHOTOENHANCEMENT OF LUMINESCENCE The general features of photoenhancement of luminescence were determined in preliminary experiments with white light. The cell suspensions used for this study were from cultures of *Gonyaulax* which, during the preceding 4 day growth period, had been illuminated during the light period with white light of 200 foot-candle intensity. During the subsequent 12-hour light period, controls received no illumination while experimental samples were exposed to illumination of various intensities and durations. All light exposures terminated at the same time. The luminescence was measured 5 to 7 hours after the end of the light period. The increase in luminescence subsequent to illumination is plotted in Fig. 4. When the data are plotted with light intensity as abscissa (upper graph), it may be seen that in all cases maximum photoenhancement occurred at about 800 foot-candles. At the temperature used (20°C.), photosynthesis also reaches a maximum at about 800 foot-candles (Haxo and Sweeney (1955)).

The magnitude of the increase in luminescence during the dark period is a function of both the intensity and the duration of illumination during the preceding light period. A linear relationship is not observed over the whole range of intensity. It is interesting to note (Fig. 4, lower graph), that 12 hours of illumination caused a disproportionately large increase in luminescence. This observation suggests that light may not be equally effective throughout the light period.

For determining the action spectrum for photoenhancement of luminescence, cells were illuminated for 12 hours with monochromatic light of different spectral regions, and the difference between the luminescence of illuminated and darkened cell suspensions was measured as described above. The light intensities were adjusted to provide equal numbers of quanta (0.24 μ einstein per minute per cm.^{2}) at each wave length band, and were in the range in which effectiveness increases approximately linearly with increasing light intensity. A small correction for deviation from linearity was

[3] This product was kindly supplied by the Hercules Powder Co., Wilmington, Delaware.

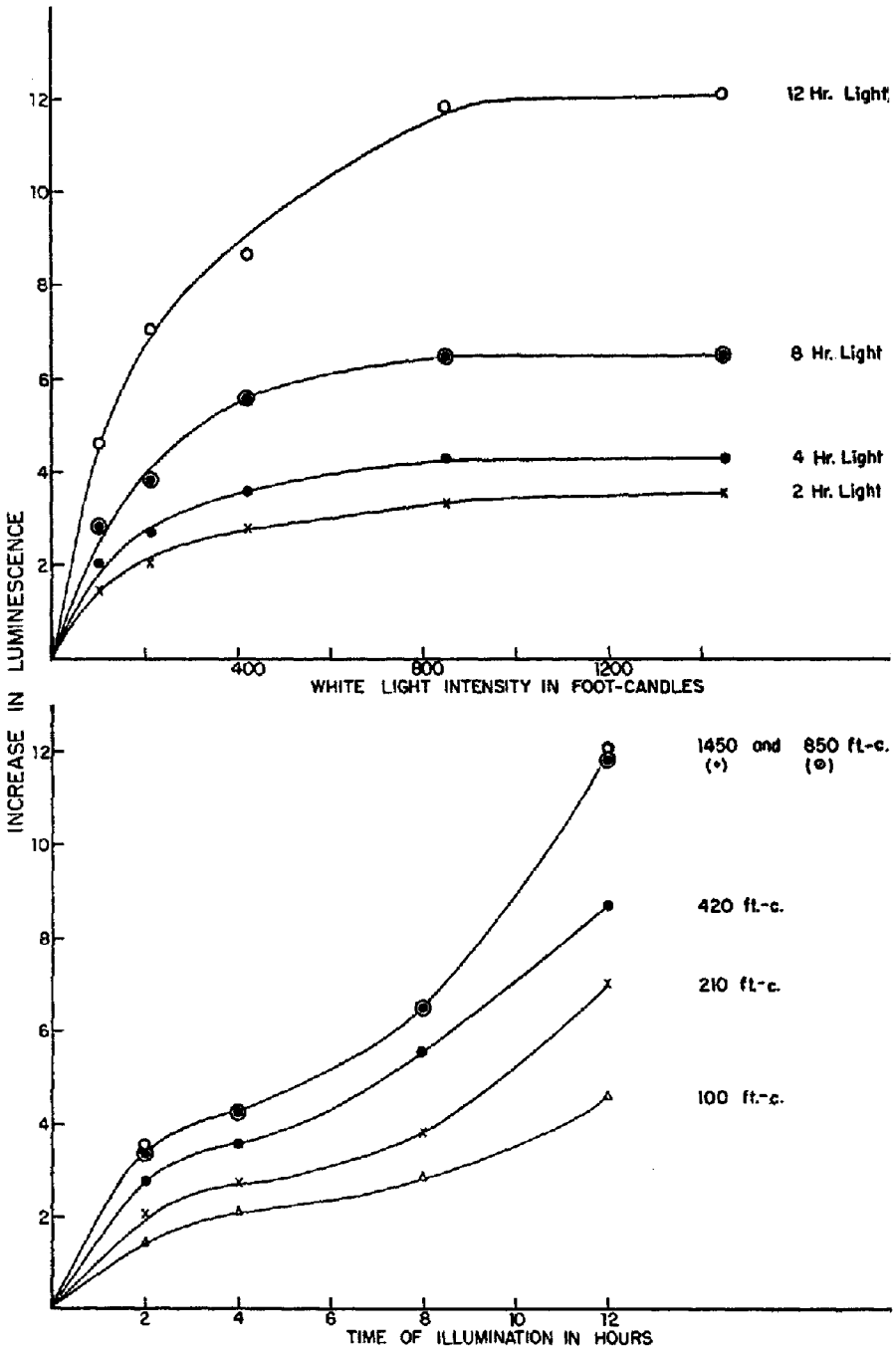

FIGURE 4. Photoenhancement of luminescence capacity of *Gonyaulax polyedra* by white light. Cell suspensions were irradiated during the day, and the luminescence was measured the following night. The ordinate represents increase in luminescence as compared with that of cell suspensions darkened during the day. The abscissas are light intensity in foot-candles (upper graph) and duration of exposure to light in hours (lower graph). Each point represents the average of three to five determinations.

applied utilizing the response curve for white light run concurrently and covering a wide range of intensities. The data so obtained are plotted as the action spectrum for photoenhancement of luminescence in Fig. 5, where they are arbitrarily superimposed at 678 mμ on an absorption spectrum for a suspension of *Gonyaulax* cells. Also included are the estimated contributions to absorption by the major pigment fractions at three wave lengths. Detailed absorption curves of the pigments in methanol from which these values were derived are shown in Fig. 6.

It is apparent from Fig. 5 that the action spectrum for photoenhancement bears a resemblance to the absorption of the cell suspension in that activity

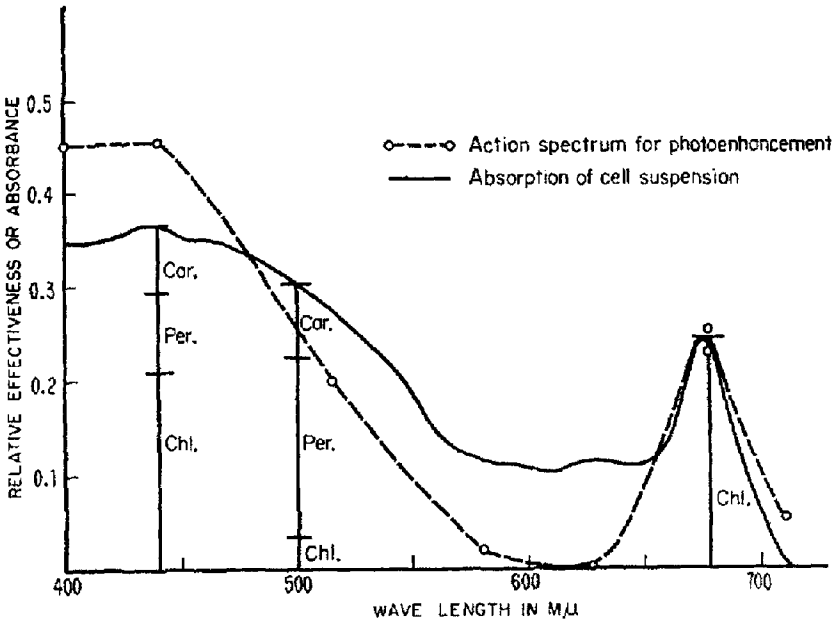

FIGURE 5. Action spectrum for photoenhancement of luminescence capacity in *Gonyaulax polyedra*. An absorption curve of a somewhat concentrated cell suspension is included for comparison, the two curves being arbitrarily superimposed at 675 mμ. The approximate contributions to absorption by peridinin (per.), other carotenoids (car.), and chlorophylls *a* and *c* (chl.) are shown here by vertical bars. The values were derived from the data for methanolic extracts presented in Fig. 6. In order to correct for the effect of the solvent, pigment absorptions at 440 mμ and 500 mμ were shifted toward longer wave lengths as follows: chlorophyll *a*, 7 mμ; chlorophyll *c*, 20 mμ; peridinin, 40 mμ; other xanthophylls, 20 mμ.

is high in the red and blue regions in which the chlorophylls absorb strongly and minimal in the regions in which chlorophyll absorption is low. Thus chlorophyll may be implicated as a sensitizer for photoenhancement of luminescence. It would appear also that carotenoids, notably peridinin, are

sensitizers; otherwise the effectiveness would certainly not be so high in the blue-green region of the spectrum in which, as shown in Figs. 5 and 6, carotenoids account for the major fraction of the absorbed light.

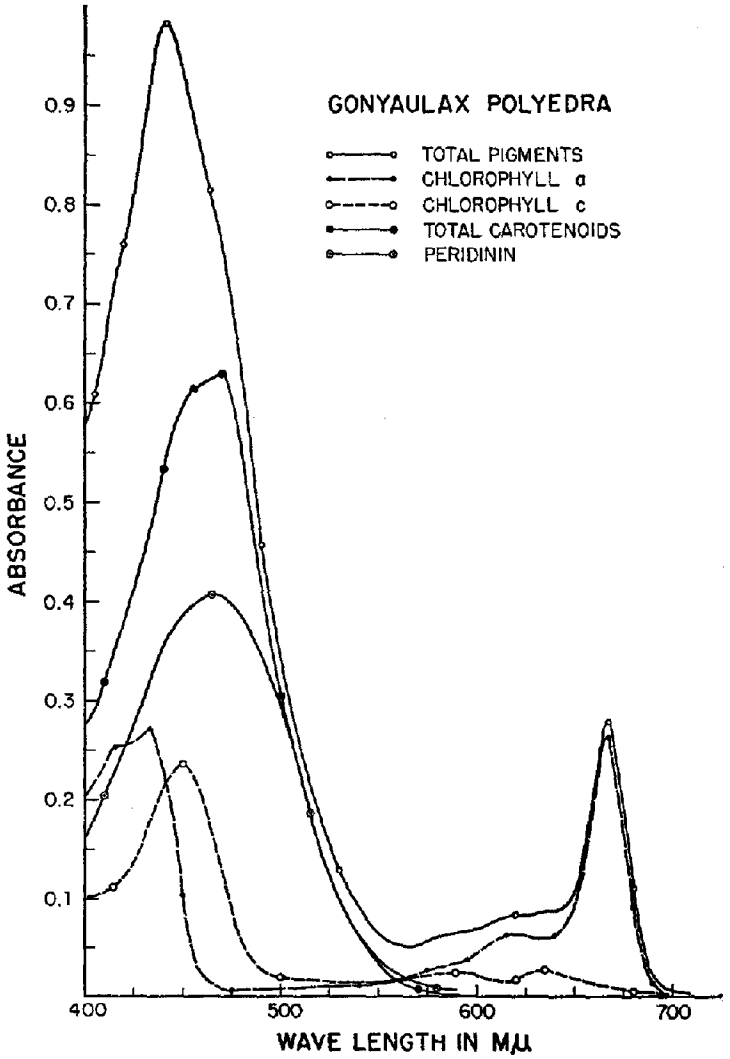

FIGURE 6. The absorption of methanolic extracts of the pigments of *Gonyaulax polyedra*.

PHOTOINHIBITION OF LUMINESCENCE As shown diagrammatically in Fig. 1, curve A, exposure of *Gonyaulax* cells to continuous light of about 800 foot-candle intensity suppresses the rhythm in luminescence, while at intermediate intensities (Fig. 1, curve B), the effect is only partial. Photoinhibition may therefore be measured accurately and conveniently during the increase

in luminescence as the difference between the luminescence of illuminated samples and that of dark controls.

The following experimental procedure was adopted: samples from a culture of *Gonyaulax* growing with alternating light and dark periods of 12 hours each were prepared at the beginning of a light period. This light period

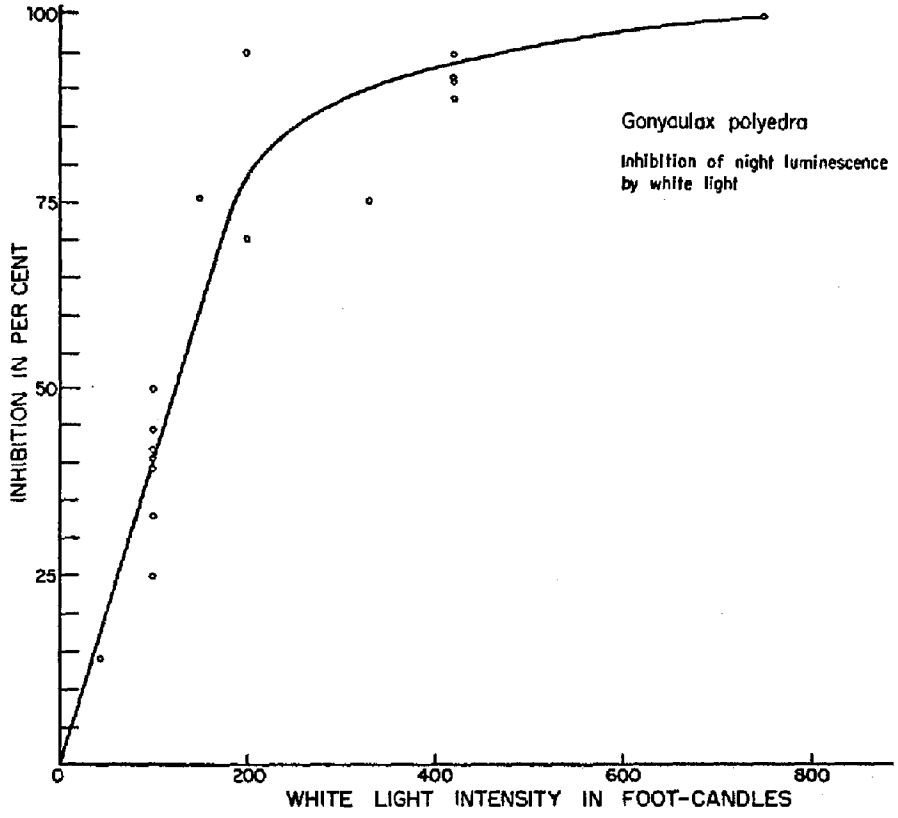

FIGURE 7. The effect of white light of different intensities on photoinhibition of luminescence capacity in *Gonyaulax polyedra*.

was prolonged by 2 hours, since it was found in preliminary experiments that such treatment increased the rate at which maximum luminescence was attained during the subsequent dark phase. At the end of this illumination period, a number of samples of the cell suspension were placed under each of the following experimental conditions: (*a*) darkness; (*b*) monochromatic light at several intensities; (*c*) white light at 1000 foot-candles as a measure of the maximum photoinhibition obtainable; and (*d*) white light at 200 foot-candles. The latter provided a measure of the sensitivity of the cells employed. Under the experimental conditions used, the maximum

luminescence in darkness was about ten times that in 1000 foot-candle white light. Photoinhibition, expressed as per cent of the luminescence of darkened controls, was found to be constant with time. The recording of luminescence in an experimental series usually required about an hour, during which time measurements of the dark controls were interspersed with those of samples which had been irradiated.

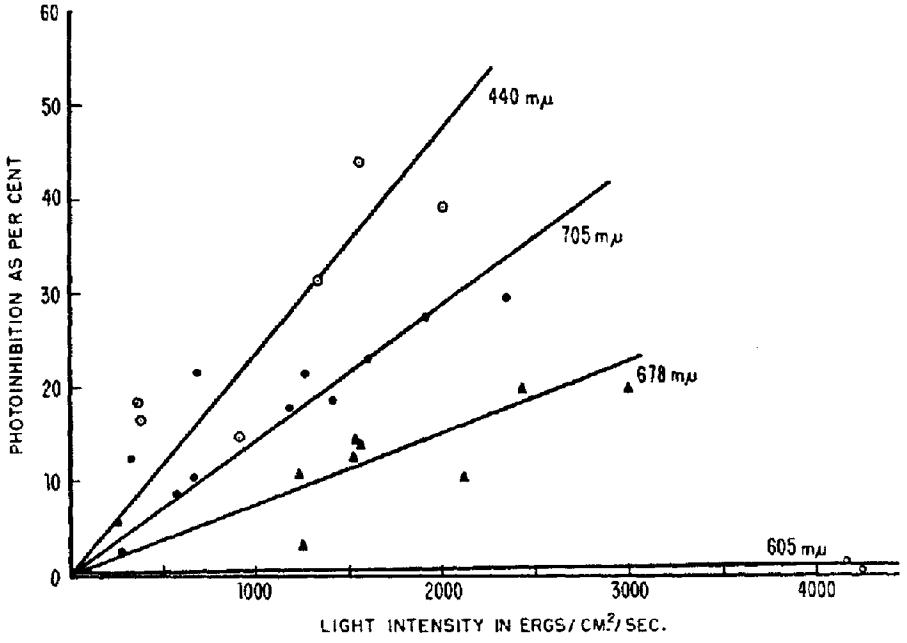

FIGURE 8. Photoinhibition of luminescence capacity in *Gonyaulax polyedra* by monochromatic light of different intensities: (⊙) 440 mμ; (○) 605 mμ; (▲) 678 mμ; (●) 705 mμ. Each point represents a single determination.

When cell suspensions were illuminated with white light of different intensities during the bright phase, the resulting inhibition of luminescence was found to be a function of light intensity (Fig. 7). Photoinhibition, like photoenhancement, is maximal at about 800 foot-candles.

Representative results for the dependence of inhibition upon the incident intensity of monochromatic light are shown for several wave lengths in Fig. 8. It may be noted that the scattering of points shown here was typical and that efforts to reduce this variability were unsuccessful. These and other similar results serve to indicate that the inhibitory effect was linear with intensity for all wave lengths studied. The per cent inhibition was then calculated for equal incident quanta at each wave length with reference to an intensity of 2000 ergs per sec. per cm.² at 440 mμ.

The action spectrum for photoinhibition obtained by plotting these values against wave length is shown in Fig. 9. The curve shows a maximum in activity at about 440 mμ and a minimum close to 600 mμ, being similar in this respect to the action spectrum for photoenhancement. A lower maximum in the red extends broadly from about 660 to 720 mμ, with an apparent peak at about 700 mμ. Activity was low but still measurable at 735 mμ, the longest

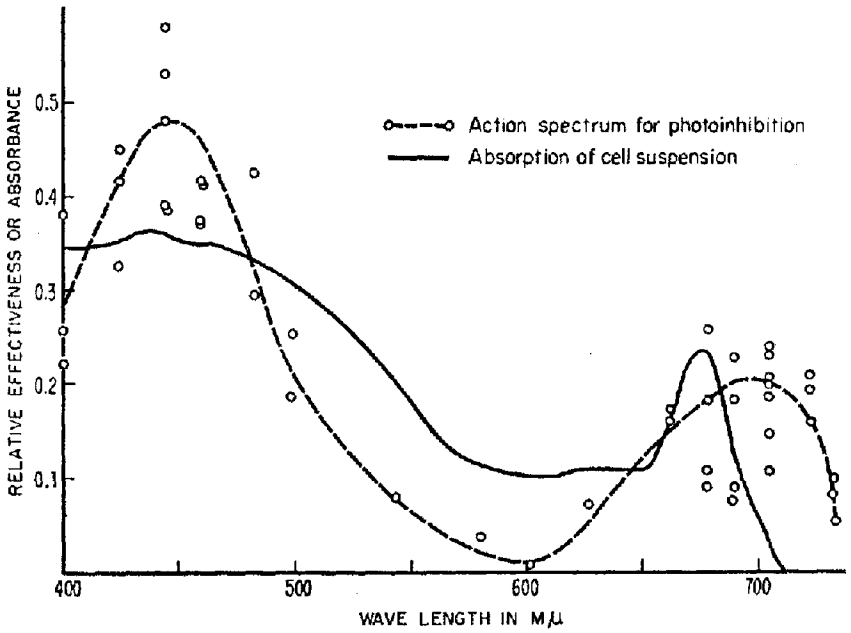

FIGURE 9. The action spectrum for photoinhibition of luminescence capacity in *Gonyaulax polyedra*. Each point is derived from a single experiment as shown in Fig. 8. The absorption spectrum for a cell suspension is included for comparison.

wave length tested. The results suggest that chlorophyll and perhaps other photosynthetic pigments are the dominant sensitizers in the spectral region 400 to 680 mμ. Because of the marked fall-off in absorption by chlorophyll *a* to negligible absorption beyond 710 mμ, we can only speculate that an unidentified pigment must be acting as the sensitizer for the inhibition of luminescence in the region of 690 to 735 mμ. This must be a minor component, since it does not appear to contribute significantly to the absorption by intact cells.

DISCUSSION

Preliminary determinations of the action spectrum for photosynthesis in *Gonyaulax* indicate that both chlorophyll and peridinin are active as sensitizers

for photosynthesis (Haxo, unpublished data). Since the action spectrum for photoenhancement of luminescence capacity implicates the same plastid pigments, it is suggested that photoenhancement is mediated through photosynthesis. This suggestion receives additional support from the observation that photoenhancement of luminiscence capacity is maximal at the same light intensity as reported previously for photosynthesis; *i.e.*, about 800 foot-candles at 20°C. (Haxo and Sweeney (1955)).

Photosynthesis is, of course, necessary for the maintenance of any photoauxotrophic cell such as *Gonyaulax*. Products of photosynthesis, such as short chain carbon compounds and energy-rich molecules, presumably enter the intracellular metabolic pool and storage depots, from which they may be later withdrawn as required for various cellular processes, including luminescence. Although the components involved in the luminescent reaction in *Gonyaulax* are not known at the present time, it is of interest to note that the biosynthesis of the components of the luminescent system apparently takes place during each cycle of the endogenous rhythm, since the levels of luciferin and luciferase in the cell are known to fall during the day and rise at night (Hastings and Sweeney (1957)).

Although between 440 and 660 mμ the action spectrum for photoinhibition of luminescence appears to be very similar to that for photoenhancement, photoinhibition cannot be related to photosynthesis in any simple way because of the activity in the far red region of the spectrum, in which absorption by chlorophyll is negligible. Although an accurate measurement was not possible, the action spectrum may actually have two absorption maxima in the red, with one peak at about 680 mμ and the other at 710 mμ. Two possible interpretations may be considered. It may be that chlorophyll and peridinin sensitization accounts for the activity between 440 and 680 mμ and that still another pigment is responsible for the activity above 680 mμ. It is also possible that the resemblance of the action spectrum for photoinhibition to chlorophyll and carotenoid absorption is fortuitous and that a different pigment present in small amounts accounts for the activity throughout the spectrum.

Light in the region of the visible spectrum beyond 680 mμ is known to produce a number of physiological effects. Notable among these is the promotion of germination in lettuce seeds by light of about 690 mμ (Hendricks and Borthwick (1954)). Effects of this type are reversed by far red light of wave lengths beyond 700 mμ. However, experiments have shown that far red light (720 to 1200 mμ) does not reverse the photoinhibition of luminescence capacity in *Gonyaulax*. Mohr (1957) has reported an effect of red light in the proximity of 700 mμ on anthocyanin formation and the inhibition of hypocotyl elongation in mustard seedlings. These effects are evident at relatively high light intensities and are not reversed by far red light. Evidence has also been presented by Kok (1957) based upon difference spectra concerning the presence, in variously colored algae and higher plants, of a pigment

which absorbs maximally at 705 mμ to 708 mμ and undergoes changes upon irradiation *in vivo*. However, it cannot be stated at present whether the photoinhibition of luminescence bears any relationship to these effects.

The action spectrum for photoinhibition in *Gonyaulax* may be contrasted with inhibitory effects in other luminescent systems. In the luminous bacterium, *Photobacterium* (Van der Kerk, 1952) and the luciferin-luciferase system of *Cypridina* (Chase and Giese (1940); Harvey (1952)), only the far blue and ultraviolet regions of the spectrum are effective, unless sensitizers such as eosin, riboflavin, or methylene blue are present. Furthermore, exposure to white light does not reduce the intensity of the luminescence of cell-free extracts of *Gonyaulax*, which are prepared from acetone powders and hence largely devoid of fat-soluble pigments. Thus, the photoinhibition of luminescence in *Gonyaulax* cells described here would not appear to be attributable to direct photolability of luciferin and luciferase.

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