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Mechanical stimulation of bioluminescence in the dinoflagellate *Gonyaulax polyedra* Stein

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Abstract: Cultures of the marine dinoflagellate *Gonyaulax polyedra* Stein were exposed to a variety of flow regimes in small tubes, pressure chambers, and vessels in which objects could be rotated. Bioluminescence was mechanically stimulated by changes in shear, acceleration, and pressure, not by constant values of these parameters. In a biological context, such stimuli would be associated with waves and other surface turbulence, with moving objects such as ships or some large marine organisms, or with close or direct contact as would occur if the dinoflagellate is a prey item. The effects of pressure are complicated by the observation that the luminescence response did not occur in the bulk of the fluid in a pressure chamber, but was confined to the liquid boundaries. The importance of luminescence at surfaces was also seen when objects were rotated in suspensions of *G. polyedra*; light emissions were restricted to regions with sharp shear gradients. These data were obtained using an image intensifier which made it possible to visualize the spatial pattern of luminescence in the various flow regimes studied. Past results obtained with photomultipliers are shown to be misleading.

Key words: Bioluminescence; Dinoflagellate; *Gonyaulax polyedra*; Image intensifier; Mechanical stimulation

Introduction

Dinoflagellates are well known for their ability to bioluminesce. Light emission occurs in two forms – as a continuous low-level glow and as rapid flashes lasting ≈ 100 ms – the intensity of which can vary on a circadian basis. Numerous parameters can trigger these emissions, including pH, temperature, electric currents, osmotic shock, mechanical stress, and various chemicals (Harvey, 1952).

The spectacular blue light seen with simple agitation of a culture flask in the dark or the motion of an object through the water is a commonly observed manifestation of mechanical stimulation of bioluminescence, the physics and physiology of which remain poorly understood despite numerous studies. In the laboratory, mechanical stimulation

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has been achieved through the generation of a shock wave from a piezoelectric cylinder (Reynolds, 1970; 1972), by flow through a capillary (Christianson & Sweeney, 1972), and by controlled pressure changes (Gooch & Vidaver, 1980; Dunlap *et al.*, 1981; Swift *et al.*, 1981; Donaldson *et al.*, 1983).

The experiments by Christianson & Sweeney (1972) utilized a coiled capillary tube in which flow rates could be controlled so as to maintain Reynolds numbers below the turbulent regime. As cultures of *Gonyaulax polyedra* were passed through the capillary, the luminescent emissions were quantified, presumably induced by the fluid shear force which was calculated to be 35 dynes $\cdot$ cm^{-2} at the maximum flow rate used. Donaldson *et al.* (1983) quantified the luminescence of *G. polyedra* and *Pyrocystis lunula* under varying pressure regimes and attributed the light emission to pressure changes. They could not, however, determine whether the magnitude of change or the rate of change was the critical parameter.

In these latter two studies, light emissions were measured with photomultipliers, which provide a quantitative measure of the luminescence output and its time dependence, but which do not provide information on the location of the emission. Thus effects restricted to the surface of the fluid, at irregularities or flow transition points, or at different positions within a shear field could not be resolved. We report here the use of an image intensifier system, which does provide spatial information, to investigate the distribution of luminescence in *G. polyedra* Stein cultures under a variety of mechanical stresses. The results clearly demonstrate that it is not the amount of shear, pressure, or acceleration that stimulates dinoflagellate bioluminescence under the conditions studied but the rate of change of these parameters.

Materials and Methods

CULTURES

Gonyaulax polyedra (strain 60 from J.W. Hastings) was grown in f/2 medium (Guillard & Ryther, 1962) with no added silicate and 10^{-5} M ferric sequestrine as iron source and chelator. Illumination was provided by cool-white fluorescent bulbs at $\approx 200\ \mu E \cdot m^{-2} \cdot s^{-1}$ on a 14 : 10 L : D cycle at 20 °C. The timing of the light : dark cycle was adjusted so that the cultures were dark-acclimated when removed from the incubator for experiments. Cultures were grown in 1 l of medium in 2-l Erlenmeyer flasks and used for experiments when cells were in early exponential growth. Cell concentrations were determined by counts of Utermohls-fixed samples in Palmer–Maloney slides. Typical cell densities were 900–1500 cells $\cdot$ ml^{-1}. The uniformity of the suspensions in the experimental containers was confirmed by cell counts of samples taken from various positions in the container.

DETECTOR

Bioluminescence was detected by means of an EMI 9912 four-stage image intensifier fitted with an S20'B' photocathode. The luminescence emitted by the organisms in suspension was directed to the input cathode of the intensifier by an f/1.4 lens with a field of view ≈ 8 in. in diameter. The output phosphor of the intensifier was viewed either by means of a conventional camera, or a SIT vidicon. With the vidicon, the luminescence could be viewed in real time on a TV monitor and simultaneously recorded on video tape for subsequent replay and analysis. This provided a resolution of ≈ 20 ms. The image intensifier was capable of providing a photon gain of $\approx 10^6$, although in these experiments, the gain was never required to be $> 10^5$. With the SIT vidicon used to observe the intensifier output, individual *G. polyedra* flashes could be seen at a gain of 5×10^3.

FLOW SYSTEMS

A variety of flow fields were achieved using the devices shown in Fig. 1. The coiled capillary (Fig. 1A) is the same device used by Christianson & Sweeney (1972), kindly provided by B. Sweeney. It was used to expose the dinoflagellates to a variable centripetal acceleration and constant shear field. Fig. 1C shows two Pasteur pipettes (Fisher Scientific No. 13–178–20A) joined together, yielding a flow tube with a 1-mm diameter narrow section that tapers to 6 mm at both ends. This configuration exposed cells to changing pressure, velocity, and acceleration. The inside diameters of one of these pipettes were 1, 1.05, 1.25, 2.1, and 6.0 mm at respective positions 0, 1.5, 3.0, 4.2, and 5.2 cm from the narrow tip. The dual pipette configuration was connected to a vertical storage cylinder (2.5 cm diameter, 60 cm high) which was filled with *G. polyedra* culture drawn from a supply beaker using a controlled vacuum. The flow rate through the pipette was thus controlled in one direction by the vacuum and that in the other direction by a combination of hydrostatic head and exit tube constriction. Flow rate through the pipette was determined by noting the change in level height in the storage cylinder through time or, alternatively, by measuring the rate of fluid leaving the system.

A small rectangular lucite box ($18 \times 1.4 \times 2$ cm) connected to a shaft was used to study acceleration and pressure effects in the absence of fluid motion. The box could be filled with culture (4.5 ml) and rotated with the shaft in either a vertical or horizontal direction. Another rotating object used in these experiments was a solid, axially symmetric ellipsoid (Fig. 1F) which was rotated in a large beaker filled with *G. polyedra* culture. This device provided variable angular velocities at different positions on its surface as it was rotated in a cell suspension.

PRESSURE CHAMBER

Fig. 1H shows a pressure chamber fashioned from a syringe plugged to form a chamber 2 cm in diameter with a volume of 8 ml. A low friction piston transmitted

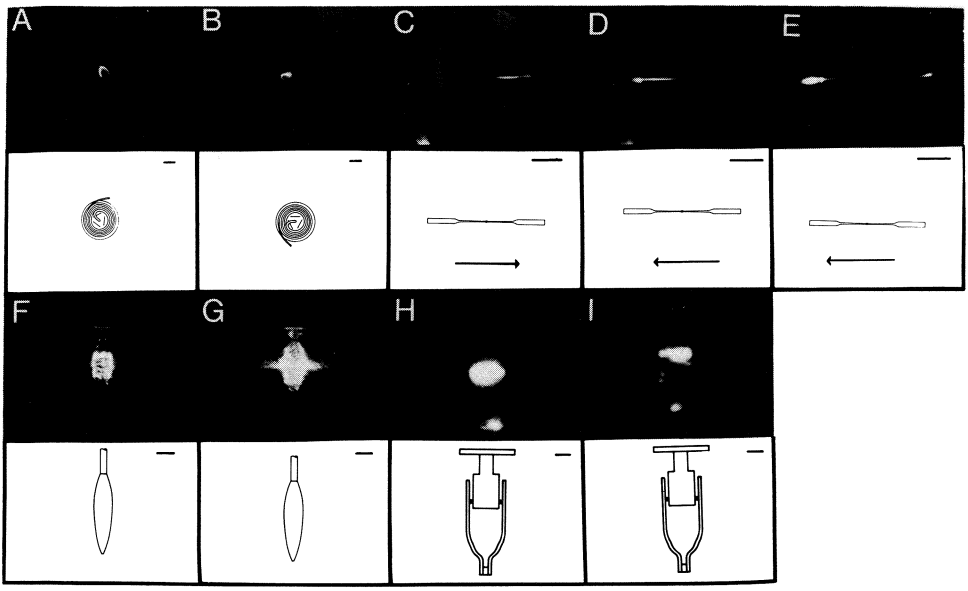

Fig. 1. Luminescent emissions for various flow configurations. In all cases, the device used in the experiment is shown directly below the photograph of the light pattern, with the relevant scale bar. (A) Flow in the spiral capillary. Flow rate is 7 ml · min^{-1}, R_e = 340, scale bar = 1 cm. (B) Light pattern from a small light-emitting diode placed at the entrance to the spiral capillary where the tubing diameter decreases abruptly, scale bar = 1 cm. Note that this photograph did not require the image intensifier and thus is optically reversed from that in Fig. A. (C) Flow through the double pipette configuration, cells moving from left to right, v = 130 cm · s^{-1}, R_e = 1300 in the narrow section, 225 in the widest portion, scale bar = 5 cm. (D) Flow in the pipette from right to left, v = 75 cm · s^{-1}, R_e = 750 and 120 in the narrow and wide sections, respectively, scale bar = 5 cm. (E) Flow from right to left, R_e = 15,000, scale bar = 5 cm. (F) Rotation of ellipsoid (radius of curvature 7.6 cm, maximum diameter 1.9 cm, 0.6 cm half diameter, scale bar = 2 cm) in *G. polyedra* suspension at 24 radians · s^{-1}. (G) Rotation of ellipsoid at 120 radians · s^{-1}, scale bar = 2 cm. Note the abrupt transition between luminescence and darkness on the surface of the object. (H) Luminescent response to pressure increase of 1 atm in the pressure chamber, scale bar = 1 cm. (I) Pressure decrease of 1 atm, scale bar = 1 cm.

pressure, either to air above a *G. polyedra* culture, or directly to the liquid surface in cases where efforts were made to remove all air. Pressure changes were made by manually adding or removing weights from the piston. To avoid the effect of shock waves, these pressure changes were applied over $\approx$0.5 s.

CALCULATIONS

From Poiseuille's formula relating parameters of fluid flow (Daily & Harleman, 1966) we can determine velocity (v), shear (S) and Reynolds number (R_e) for the various capillary devices used in this study. The pressure p and velocity variation of a fluid with density ρ in a tube of varying radius can be determined by means of Bernouilli's equation

$$\tfrac{1}{2}\rho v^2 + p = \text{constant}, \qquad (1)$$

where it is assumed that the tube is horizontal and the flow frictionless. The centripetal acceleration a_c experienced by an object within the spiral capillary or the rotating lucite chamber can be calculated as

$$a_c = w^2 r, \tag{2}$$

where w is the angular velocity of rotation and r is the distance from the axis of rotation.

Results

When *G. polyedra* cultures were pumped through the spiral capillary configuration shown in Fig. 1A, the luminescent response (Fig. 1A) did not occur in the uniform section of the capillary, but was initiated at the transition point between the tube leading from the culture (5 mm i.d.) and the capillary itself (0.86 mm i.d.). The light decreased in intensity rapidly as the cells proceeded around the spiral and was undetectable after one-half revolution. The image shown is from a flow rate of 7 ml $\cdot$ min^{-1}, corresponding to a maximum velocity of $\approx$40 cm $\cdot$ s^{-1}, a maximum shear of 19 dynes $\cdot$ cm^{-2}, and a Reynolds number of 340. The centripetal acceleration thus varied between 1.6 g near the center of the spiral to 3.2 g at the outer boundary. Since the spiral tube was 61 cm in length, the cells experienced a change in acceleration of $\approx 1\, g \cdot s^{-1}$. The same luminescence emission pattern was seen at higher flow rates as well.

Fig. 1B shows the light pattern when a point source (a small light emitting diode or LED) was placed at the constriction at the entrance to the capillary. The image intensifier was not used for this photograph, so the image is reversed, but the same general pattern seen with *G. polyedra* is evident, including the decay of intensity within one-half revolution.

The second "capillary" configuration tested was with two pipettes joined together at their narrowest point (Fig. 1C). When flow was laminar in the narrow section, light was only emitted by the cells downstream of the joint between the two pipettes (Fig. 1C,D). This occurred even when the Reynolds number was as low as 160 in the narrowest section of the tube. At much higher flow rates (R_e = 15,000), light was emitted at both ends of the configuration in the region where the pipettes become widest (Fig. 1E). To determine whether light piping was occurring, a LED was placed in the neck of the pipette, but only a single point of light was observed. Furthermore, to determine whether the absence of luminescence in certain sections of the tube was due to *G. polyedra* fatigue following their stimulation in upstream sections of the tube, one-half of the pipette configuration was used, allowing exiting cells to fall into a beaker placed in the field of the image intensifier. The intense light observed as the cells emerged and fell into the liquid showed that fatigue was not responsible for the lack of luminescence observed in certain sections of the tube.

RESPONSE TO ROTATION

A small rectangular lucite chamber mounted on a shaft was filled with *G. polyedra* culture and rotated horizontally and vertically. With the shaft in a horizontal orientation, as with an airplane propeller, the cells in the chamber experienced rapid changes in hydrostatic pressure during rotation as well as a constant centripetal acceleration at each point in the fluid, but there was no shear since there was no fluid flow. A pressure change of ≈ 0.15 atm $\cdot$ s^{-1} occurred with a rotation rate of 30 radians $\cdot$ s^{-1}. No luminescent response was observed at velocities between 10 and 100 radians $\cdot$ s^{-1}. When the shaft was rotated about a vertical axis, as with a helicopter propeller, the cells in the chamber experienced only centripetal acceleration – no shear or hydrostatic pressure changes. At steady state (i.e., uniform angular velocity and thus constant accelerations) no luminescent response occurred for accelerations in excess of 100 *g*. However, luminescence was stimulated by suddenly starting or stopping the rotation.

RESPONSE TO ROTATING BODIES

The ellipsoid shown in Fig. 1F was rotated in a large beaker of *G. polyedra* cells. Rotational speed could be increased at a controlled rate or preset to initiate rotation rapidly when a switch was closed. Observations showed that by far the major luminescent response occurred when the motion was initiated. When angular velocities were sufficiently high, the luminescence persisted for a short time after the final velocity was achieved. Fig. 1F,G show that the response was restricted mainly to the surface of the rotating object and to a region extending outward from the equator. The general pattern of light emission can be characterized by intense luminescence where the elipsoid was widest and velocities highest, and weak luminescence where the surface velocities were lowest at the top and bottom of the object (Fig. 1F,G). The Reynolds number of the flow at the surface of the ellipsoid is a function of the distance from the central axis, increasing with the square of that radius for a given angular velocity. With angular velocities of 24, 40, 56, and 120 radians $\cdot$ s^{-1}, the boundary where the abrupt transition from low to high luminescence occurred was associated with Reynolds numbers of 2500, 2460, 2650, and 2350, respectively.

RESPONSE TO PRESSURE CHANGES

The chamber shown in Fig. 1H was used to investigate the luminescence response of *G. polyedra* to pressure change (one atmosphere, the increase or decrease occurring over 0.5 s and remaining static thereafter). As weights were added or taken away from the piston, the dominant response was luminescence at the top and bottom surfaces of the chamber, the former being the site of the most intensity (Fig. 1H,I). The same pattern was observed when a small amount of air was present between the piston and the liquid as when all air was removed, although more consistent results were observed with no air. Luminescence was stimulated by both pressure increases and decreases, with the

most light being emitted following increased pressure. In all cases, the luminescence was observed when the pressure changed; other than the occasional flashes of individual cells, there was no light from the chamber once the pressure stabilized.

Discussion

Dinoflagellate bioluminescence can be mechanically stimulated by shear, acceleration, and pressure, although in the context of our experiments, it is the temporal change of these parameters that is critical, not their magnitude. This conclusion is made possible in large part through the use of an image intensifier for monitoring the light emission. By observing the precise locations where luminescence occurs in a capillary tube, pressure chamber, or near a rotating object, it is possible to link the observed light emissions to one or two possible physical stimuli. Taken together, the results from the experiments in these different types of devices begin to define a consistent response of dinoflagellates to changes in their physical environment. Previous studies of this type have used photomultipliers to record emissions, but these devices provide no information on the location of the luminescence and as will be discussed below, have led to erroneous conclusions. The limitations of photomultipliers are exacerbated by the dominance of surface effects whereby, as shown here, luminescence occurs at the boundaries of a liquid in a chamber or at the surface of rotating or moving objects.

Cells travelling through the spiral capillary configuration used by Christianson & Sweeney (1972) and by us (Fig. 1A) experienced several mechanical stresses. At the entrance to the spiral from the supply tube, the abrupt change in tube diameters and flow direction resulted in a discontinuity sufficient to stimulate luminescence. As demonstrated in Fig. 1A, the emitted light decayed rapidly, but did extend one-half revolution beyond the discontinuity through a combination of the 50–100-ms lifetime of the *G. polyedra* luminescent flashes (Krasnow *et al.*, 1981) and a light piping effect (Fig. 1B). Once the cells were beyond the discontinuity at the entrance to the spiral, their paths around the coils exposed them to a variable centripetal acceleration and a constant shear field along streamlines, all in laminar flow. The lack of luminescence in the spiral downstream of the discontinuity (Fig. 1A) indicates that, in the range of the parameters tested, constant shear does not trigger *G. polyedra* bioluminescence, nor does variable acceleration at the rate of $1\ g \cdot s^{-1}$.

It should be stressed that our results using the image intensifier offer a different perspective from those of Christianson & Sweeney (1972) who used a photomultiplier on the same spiral capillary. The luminescence they observed with *G. polyedra* was assumed to originate within the six coils of the spiral, possibly in response to the constant shear field, which they calculated to be 35 dynes $\cdot$ cm^{-2}. Since our experiments were within the range of flow rates they used and the capillary configuration was identical, we conclude that the light measured by their photomultiplier was all from the discontinuity at the spiral entrance and therefore was not in response to constant shear.

To further study the effects of shear, the double pipette configuration shown in Fig. 1C was employed. At velocities where flow was laminar in the narrowest portion of the tube, luminescence was only detected in the region where the diameter gradually increased (Fig. 1C,D). The luminescence as the pipette bore widened occurred as the velocity of the fluid decreased and the pressure correspondingly increased (Eqn. 1). Upstream, the tube narrowed in the direction of the flow, leading to a velocity increase and a pressure decrease. If the flow were frictionless, the pressure differences between the largest and smallest sections of the pipette would be $\approx 10^{-2}$ atm, occurring in 0.1 s at a flow velocity of 37 cm $\cdot$ s^{-1}, calculated using Eqn. 1. However, the viscosity of the seawater introduces viscous pressure losses in the tube which have the net effect of making the pressure decrease greater than Bernouilli's equation would predict as the pipette narrows, whereas the pressure increase would be smaller than the equation indicates as the pipette widens. We calculate these viscous pressure losses to be approximately half of the Bernouilli pressure changes in the dual pipette configuration.

The arguments above indicate that the magnitude of the net pressure decrease as the tube narrowed was larger than the pressure increase as the tube widened further downstream, yet the luminescence was consistently observed in the latter sections. Stated differently, pressure decreases were far less effective in stimulating luminescence than pressure increases. Only when the fluid velocity was increased to the point where flow was clearly turbulent (Reynolds number 15,000; Fig. 1E) did luminescence appear in the pipette as it narrowed.

The dominance of luminescence as pressure increased during laminar flow in the dual pipette configuration might be related to the fact that fluids converge and accelerate more easily than they diverge and decelerate. This might be reflected in a non-symmetrical flow where the tube widens, possibly with an altered velocity profile in which the flow near the tube walls tries to reverse direction. The resulting shear gradients would be very large indeed and likely to cause luminescence. In this instance, the luminescence would be directly caused by shear, although the indirect cause would be the increasing pressure.

It is clear that a detailed hydrodynamic study would be needed to clarify this mechanism. However, for the purposes of this study, the experiments with the dual pipette configuration indicate that luminescence occurred when pressure and shear were changing, with the most intense emissions associated with regions where the pressure changes were positive.

In an effort to separate the effects of pressure and shear, the *G. polyedra* culture was placed in a lucite chamber that could be rotated. Since there was no fluid motion within the chamber, there was no shear. After a very gradual increase in rotational velocity (to avoid luminescent fatigue), the cells were exposed to constant centripetal acceleration and variable hydrostatic pressure when the rotation was about a horizontal axis, but only centripetal acceleration when the rotation occurred about a vertical axis. No luminescence was observed at constant angular velocities in either orientation, demonstrating that neither constant acceleration on the order of 100 *g* nor pressure changes

of 0.05–0.5 atm · s^{-1} could stimulate the cells. Since these pressure changes are similar to those calculated for the dual pipette flow experiments, we conclude that changes in shear were responsible for the observed luminescence in the pipette (Fig. 1C–E). A second observation from the rotating chamber is that luminescence was only seen with abrupt starts and stops – in effect that rapid changes in acceleration (on the order of tens $g \cdot s^{-1}$) triggered the emission. Changes in acceleration were also associated with flow around the spiral capillary, but those changes ($1\ g \cdot s^{-1}$) were apparently too small to elicit a response.

Although pressure changes from 0.05 to 0.5 atm · s^{-1} did not stimulate luminescence in both the dual pipette and the rotated chamber, larger changes on the order of 2 atm · s^{-1} did cause luminescence in the pressure chamber of Fig. 1H. This occurred whether air was present between the piston and the fluid or not. However, the luminescence was observed only at the boundaries of the fluid, and then more intensely at the surface than at the bottom. The intensity of the emissions was consistently higher for pressure increases than for decreases. Cells in the bulk of the fluid experienced the pressure changes but did not luminesce, suggesting that some reflection of a pressure wave or other surface effects might be involved at the surfaces.

Our results should be viewed in the context of two others that examined the effects of pressure on luminescence. Gooch & Vidaver (1980) studied the detailed kinetics of *G. polyedra* luminescence following large (560 atm) pressure changes. A sharp flash of light was associated with the pressure increase which they attributed to the combined effects of absolute pressure on chemical reaction rates and shear from the mechanical shock wave travelling through the pressure chamber. Light was also emitted from a pressure decrease of similar magnitude, but, as we observed as well, the intensity was lower than that associated with the increase. Following the initial pressure increase, Gooch & Vidaver (1980) found that luminescent emissions continued for several minutes, the kinetics being regulated by the absolute pressure. This is in conflict with our observations that constant pressure does not trigger luminescence in *G. polyedra*, but we used much lower pressures and focused on mechanical stimuli that perturb the cell physically, whereas they used pressures sufficiently high to affect the chemical reactions involved in the luciferin/luciferase system.

Another study that focussed on the effects of pressure on dinoflagellate bioluminescence was that of Donaldson *et al.* (1983), who concluded that a pressure change rate of at least 1 atm · s^{-1} was required to stimulate luminescence in *G. polyedra*. Pressure increases and decreases were both effective, but emissions from decreases were the most intense (the opposite of our conclusion). Our results with a similar pressure chamber but with luminescence detected using an image intensifier rather than a photomultiplier indicate that the response to the pressure changes was occurring at the boundaries of the liquid – in effect that surface effects were dominating the response. The lack of luminescence in the bulk of the liquid during the compressions or decompressions is an important observation that suggests to us that the light observed may only be an indirect effect of the pressure change. We clearly cannot say that the specified

pressure changes directly caused the response, since the cells in the bulk of the liquid experienced those pressure changes but did not luminesce. Cells near the fluid boundaries, especially at the surface where luminescence was the brightest (Fig. 1H,I), may have been exposed to reflected pressure waves, to the formation or collapse of bubbles resulting in a localized region of increased shear, or to some other surface effect. We have observed (data not shown) that 1-mm diameter bubbles readily stimulate bioluminescence as they move through a *G. polyedra* culture. For now, we must view our own pressure data and probably also that of Donaldson *et al.* (1983), as inconclusive but suggestive of the need to study luminescence in the context of the physics and chemistry of liquid surfaces.

Another example of the importance of surfaces is seen when objects are rotated in suspensions of *G. polyedra*. Fig. 1F,G demonstrate our typical observation that luminescent intensity was brightest on the surface of a rotating ellipsoid, falling off rapidly in the adjacent fluid. The emissions were most intense when the rotation was initiated, but at sufficient angular velocities, persisted for a short time after a steady speed was attained. The decrease in luminescence over time was probably the result of cell "fatigue". The luminescence was brightest on the surface of the object, but also extended out into the fluid in the equatorial region (Fig. 1G). This is clearly a complex flow field that is not easily quantified given the fluid's confinement within a beaker. However, at a qualitative level, we believe that the luminescent pattern resulted from fluid being propelled outward at the equator, with replacement flows along the surface of the elipsoid from above and below. Cells thus travelled rapidly from low shear regions in the body of the fluid to a high shear environment at the surface of the ellipsoid. Since the rotational velocities were highest at the equator where there was a convergence of the flows from the two "hemispheres" of the ellipsoid, the highest changes in shear occurred in that region, and that was where luminescence was most visible. We would likewise argue that the general effect of luminescence at the surface of the object (i.e., independent of the pumping at the equator) was due to cells being drawn into the high shear gradients associated with boundary layer flow at that surface.

These observations suggest once again that change in shear stimulates luminescence. Since the cells were undoubtedly "fatigued" by the rapid fluid flow, their response to constant shear once the flow had stabilized could not be evaluated in this experiment. However, experiments using the coiled and straight capillaries described earlier did allow a wide range of constant shear fields to be examined, none of which stimulated a luminescent response.

One interesting aspect of the rotating object experiments is that there were typically two regions of luminescence, distinguished by their high or low emission intensity. The areas with little or no luminescence were at the top and bottom of the rotating objects where surface velocities were lowest; the brightest emissions were in the central, high velocity section. The boundary between these regions consistently occurred at Reynolds numbers slightly > 2000. We recognize that these values for a rotating object may not necessarily signify the boundary between laminar and turbulent flow as would be the

case in a pipe or tube, but it is possible that the dinoflagellate luminescence marks the region where shear increases dramatically as laminar flow breaks down.

The general conclusion from all of our experiments is that it is the change in shear or acceleration (and possibly pressure) that mechanically stimulates dinoflagellate luminescence, not constant values of these parameters. In the dinoflagellate's environment, such stimuli would be associated with waves and other surface turbulence, with moving objects such as ships or some larger marine organisms, or with close or direct contact as would occur if the dinoflagellate is a prey item. In this latter context, one of the suggested ecological advantages of bioluminescence (Tett & Kelly, 1973; Porter & Porter, 1979) that has received increasing support from experimental studies (Esaias & Curl, 1972; Buskey *et al.*, 1983) is that bioluminescent emissions reduce grazing impact on the dinoflagellate. The response by the zooplankton predator to the luminescence has been shown to be a change in swimming behavior, from the slow speed characteristic of grazing to high speed swimming bursts which remove the animal from the light source (Buskey *et al.*, 1983). An important unknown is whether this "startle" response is to the dinoflagellate's natural flashing frequency or to light triggered by the mechanical disturbances created by the swimming and feeding behavior of the zooplankter itself. A second unknown is whether the mechanical stresses associated with either swimming or capture and feeding are sufficient to trigger a luminescent flash. To resolve this issue, the image intensifier was used by Buskey *et al.* (1985) to monitor a culture of *G. polyedra* into which copepods were placed. Their observations indicated that the most intense dinoflagellate emissions were associated with the feeding activities of the copepods, and that relatively little stimulation occurred during routine swimming. What remains to be seen is whether it is the capture motions of the copepod's appendages that trigger the luminescence from nearby dinoflagellates or just the direct contact that occurs when a cell is pulled close to the animal and ingested. Measurements are now available of the appendage velocities, setae spacings, and beat frequencies of feeding copepods (Koehl & Strickler, 1981). Our work has implicated the change of shear, acceleration or possibly pressure as the causes of mechanically stimulatible luminescence in a dinoflagellate, but there is now a need to use these techniques to determine the thresholds of those responses for comparison with the shear fields of feeding copepods. It may well be that luminescent flashes can minimize zooplankton feeding activity in dinoflagellate assemblages and cause the rejection of those that are actually captured as well.

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