The photochemical reaction model is represented in Fig. 1. The radical concentration is fed by the photolysis of ozone and removed mainly by radical-radical collisions that form water or hydrogen peroxide. The cycle shown in the center of Fig. 1 serves as a sink for carbon monoxide, and the outermost cycle both removes methane from the atmosphere and produces formaldehyde. The principal loss mechanism for formaldehyde is

$$OH + H_2C{=}O \rightarrow H_2O + CHO \quad (15)$$

The steady-state concentration of hydroxyl and hydroperoxyl radicals is given by

$$[HO_2 + OH] = \left\{ \frac{(2[O^1D]\,[H_2O]\,K_3)}{K_{12}} \right\}^{1/2}$$

and the ratio of hydroperoxyl to hydroxyl radical is given by

$$[HO_2]/[OH] = ([CO]\,K_5 + [O_3]\,K_4 + [CH_4]\,K_8)/([NO]\,K_7)$$

where I have assumed $K_{13} = 2K_{12}$ to simplify the analysis. Values of K_{12} and K_{13} are listed in Table 1 as a function of the time of day for a summer latitude of 34°. The ratio depends inversely on the value of K_7, which is not accurately known. For the calculations in Table 1, I chose an intermediate value from the literature estimates that range from 5×10^{-11} to 2×10^{-15} cm^3 molecule^{-1} sec^{-1} (*18*).

The daytime concentration of the methylperoxyl radical is proportional to the concentration of the hydroxyl radical and is well approximated by

$$[CH_3O_2] = 33\,[OH]$$

The concentration of the methoxy radical is quite small, owing to its fast removal by molecular oxygen (reaction 11).

On the basis of the hydroxyl radical concentrations given in Table 1, a carbon monoxide lifetime in the surface atmosphere can be calculated as follows:

$$\tau = 1/([OH]_{av} \times K_5) \cong 5.4 \times 10^6 \text{ sec} \cong 0.2 \text{ year}$$

where $[OH]_{av}$ is the average concentration of the hydroxyl radical during the day. This value of τ is in harmony with a recent experimental value (*19*).

With Junge's (*6*, pp. 113–124) model of particulate size distribution in the surface atmosphere, the collision frequency between atmospheric molecules and particulates is not more than 0.09 sec^{-1} over the continent and much less than this value over the ocean. Combining this upper limit for the collision frequency with experimental values for the fraction of surface collisions that result in the destruction of the gaseous species, which range from 3×10^{-3} (*20*) to 8×10^{-5} (*21*) for hydroxyl radicals, we find that the loss rate for hydroxyl radicals due to collisions with particulates may approach 3×10^{-4} sec^{-1}. Loss rates for other radicals should be similar if not slower. Since this loss rate is significantly less than the gas phase radical-radical loss rate (reactions 12 through 14), atmospheric particulates are not important in this photochemical model.

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16 February 1971; revised 3 May 1971

Light Production in the Luminous Fishes Photoblepharon and Anomalops from the Banda Islands

Abstract. *The unresolved mechanism of light production in* Photoblepharon *and* Anomalops *has been reinvestigated in fresh and preserved material. Based on biochemical evidence obtained with emulsions and cell-free extracts of the organs, especially the stimulation of light with reduced flavin mononucleotide, and on electron microscopy of organ sections showing the presence of numerous bacteria, we conclude that the light is produced by symbiotic luminous bacteria. Because of the continuing failure to cultivate the luminous bacteria and because of their morphology, we suggest that the bacteria are of a primitive type called bacteroids.*

Although bioluminescence is a very common characteristic of deepwater fishes, some shallow-water fishes are also luminous. Among the most famous are *Photoblepharon palpebratus* and *Anomalops katoptron*, which are indigenous to the Banda Sea at the eastern end of the Indonesian Archipelago. The flashes of light produced by these fishes at night are so spectacular that they have attracted the attention of students of bioluminescence for some 50 years. However, due to the remoteness of the area, few studies have been carried out.

Studies by Vorderman (*1*), Steche (*2*), Harvey (*3*), Haneda (*4*), and Bassot (*5*) have shown that the light is emitted from a pair of large elliptical organs, each lying in a suborbital depression. Each organ is attached at the dorso-anterior edge by a small piece of cartilaginous tissue. The face of the organ, which emits light continuously, is cream-colored, whereas the opposite face is nearly black owing to a pigmented cell layer. In *Anomalops*, the light is

extinguished by rotation of the entire organ along its long axis so that the luminous face is turned down and toward the body, presenting the pigmented face to the outside. In *Photoblepharon*, however, the light is extinguished by a black fold drawn up over the organ. The histology of the light organs in the two species is very similar. Each organ is filled with many tubes and blood capillaries running parallel to each other from the pigmented base to the light-emitting face. The tubes are polygonal and arranged in a rosette around the capillaries. The tubes contain bacteria (*3*, *5*). However, neither Harvey (*3*) nor Haneda (*4*) could obtain a culture of luminous bacteria from the organs, and this failure has left unresolved the origin of light in these fishes.

We collected *Photoblepharon* and *Anomalops* a short distance offshore at the Banda Islands at night from a boat, using hand nets in waters 4 to 5 m deep. *Anomalops* swam in schools of 20 to 50 near the surface, blinking rapidly as they swam. *Photoblepharon* swam either singly or in pairs, darting in and out from among the rocks and corals. They came to the surface only occasionally, and they extinguished their light only infrequently. Thus, the light shone almost continuously with constant intensity. Neither species was observed during the day. The natives of Banda call *Photoblepharon, "ikan leweri batu,"* or the "fish that lives among the rocks," and *Anomalops, "ikan leweri air,"* or the "fish that lives in the water." Both species were more abundant before moonrise or after the moon had set or on moonless nights.

Living specimens were placed individually in large glass jars containing seawater and then examined in the dark. Figure 1 shows a swimming *Photoblepharon*. The exposed light organ is prominent beneath the eye. In a specimen of *Photoblepharon* 80 mm long from tip of snout to tail base and 30 mm in depth of body, the light organ measured 9.1 mm in the long axis, 5.6 mm in the short axis, and 1.0 mm in thickness. In a specimen of *Anomalops* 94 mm long from tip of snout to tail base and 32 mm in depth of body, the corresponding measurements were 11.0 by 4.0 by 1.0 mm. These were the most common sizes found. When *Photoblepharon* and *Anomalops* were moved into a semi-darkened room, both displayed their luminous organs continuously and the light intensity appeared to be constant. When *Anomalops* was moved to a lighted room, the light organ was almost immediately rotated 120° to 135° so that the light-emitting surface moved out of view, thus cutting off the light. When *Photoblepharon* was moved from a semi-darkened to a lighted room, the fish usually did not occlude its light. When both fishes were returned to the semi-darkened room, *Anomalops* displayed its light immediately, whereas *Photoblepharon* showed little change in luminescence. They responded to a short flash of light in the same manner as they did to constant light. Handling of the fishes did not seem to affect either their blinking or the light intensity.

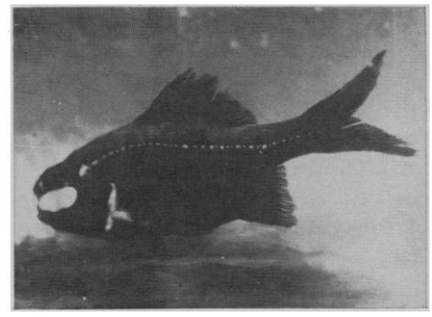

Fig. 1. Photograph of a *Photoblepharon* swimming with its light organ displayed. The lateral line system and gill opening are reflective but not luminous. About one-third actual size.

For the chemical and bacteriological studies, specimens were used immediately after being caught. In preparing luminous emulsions of the light organ, we gently ground four organs in approximately 5 ml of seawater in an all-glass homogenizer. In the chemical tests, a few milligrams of cofactor were dissolved in a few milliliters of seawater and the solution was then immediately mixed with the water extract. Bacteriological studies were performed with two different culture media. The first contained, in 1 liter of water, 30 g of NaCl, 5 g of peptone, 1 ml of glycerol, 15 g of agar, and crude aqueous extract of squid, with *p*H adjusted to 7.2 to 7.4. The second contained 30 g of NaCl and 23 g of nutrient agar in 1 liter of water, with *p*H adjusted to 6.8. For the electron microscopy, the detached photophores were placed in 10 percent formalin in seawater. After about 2 months, the photophores were washed with distilled water and fixed in 1 percent osmium tetroxide for 1.2 to 2.0 hours. The tissues were then dehydrated through a graded series of acetone as follows: 30, 40, 50, 60, 70, 80, 85, 90, 95, and 100 percent. Tissues were embedded in Epon 812, which consisted of a mixture of A and B (5:5), by the method of Luft (*6*). Ultrathin sections cut with a JUM type 5B ultramicrotome were examined in a JEM type 7A electron microscope. For the bacterial luciferase test, air-dried photophores desiccated over silica gel were used.

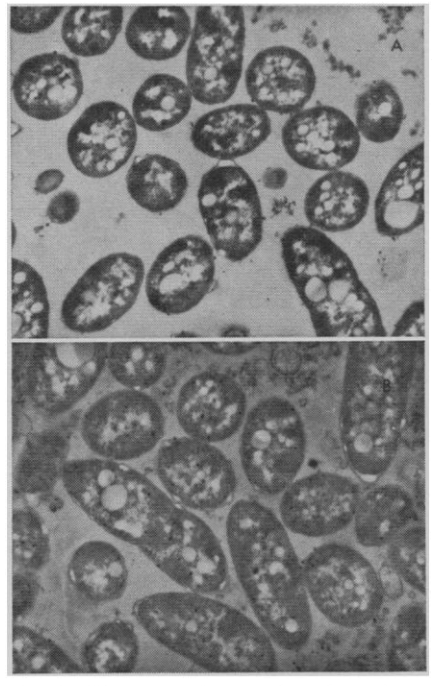

Fig. 2. Electron micrographs of sections through light organs showing bacteria. (A) *Photoblepharon*; (B) *Anomalops*. Magnification, × 20,000.

Luminous emulsions prepared from light organs quickly became dark when allowed to stand, except for a layer at the top 2 to 3 mm thick. When the darkened emulsion was shaken in air, the luminescence was completely restored. This procedure could be repeated many times with the same result. The emulsion retained its ability to luminesce for 6 to 8 hours. Raising the temperature slowly caused a gradual decrease in light intensity until at around 50°C the emulsion became completely and irreversibly dark. Lowering the temperature to around 26°C did not restore the luminescence. Light intensity appeared optimum between 23° to 26°C. When an emulsion was diluted with fresh water, luminescence was quickly extinguished. Dilution with seawater decreased the light intensity in proportion to the volume of seawater added. When a luminous organ was ground in fresh water, the light was immediately extinguished. Air-dried organs did not luminesce when moistened with water. Tests for the presence of

the luciferase-luciferin (enzyme-substrate) reaction in the organs were also performed on cold- and hot-water extracts. The cold-water extract (luciferase) was prepared by grinding four fresh organs in about 5 ml of water. The hot-water extract (luciferin) was prepared by grinding four fresh organs for 1 minute in about 5 ml of boiling water and cooling it as quickly as possible in cold water. When mixed the two extracts gave no light, indicating the absence of the luciferase-luciferin reaction. Attempts were also made, with negative results, to stimulate light emission from dark, cold-water emulsions by adding the following cofactors: adenosine 5′-monophosphate, adenosine 5′-diphosphate, adenosine 5′-triphosphate, reduced nicotinamide-adenine dinucleotide, reduced nicotinamide-adenine dinucleotide phosphate, and coenzyme A.

However, successful results in evoking light were obtained later with cell-free extracts of air-dried organs which were prepared by homogenizing two organs in 5.5 ml of distilled water. After the homogenate was centrifuged at 10,000*g* for 10 minutes at 2°C, 2.5 ml of the supernatant was mixed with 0.5 ml of a $5.84 \times 10^{-4} M$ solution of reduced flavin mononucleotide (FMN) plus a trace amount of *n*-dodecyl aldehyde. Fourteen such extracts were prepared from a total of 28 organs. Light was recorded with a photomultiplier photometer from all extracts. Controls containing approximately the same concentration of reduced FMN and *n*-dodecyl aldehyde plus known amounts of bacterial luciferase (Worthington Biochemical Corp., Freehold, N.J.) also emitted light.

Numerous unsuccessful attempts were made to culture luminous bacteria from *Photoblepharon* and *Anomalops* light organs. However, luminous bacteria isolated from the light organs of freshly collected pony fishes (*Leiognathus* sp.) grew well on the same medium.

Electron micrographs (Fig. 2) show large numbers of bacteria shaped like a rod or prolate spheroid 2 to 3.3 μm long and about 1 μm thick. The bacteria in both cases resemble in ultrastructure a gram-negative bacterium.

Our results demonstrate fairly conclusively that the origin of light in these fishes is due to symbiotic luminous bacteria. The character of the fish luminescent system seems virtually identical with that of the known bacterial system. These similarities include dependence on oxygen concentration, extinction temperature for luminescence of around 50°C, extinction of a luminous emulsion on dilution with fresh water but not seawater, absence of luminosity in desiccated organs moistened with water, and negative luciferase-luciferin reaction. Practically the same results were obtained by Harvey (*3*), who concluded that the luminescence was due to symbiotic luminous bacteria. Harvey (*3*) also found the luminescence to be inhibited by cytolytic agents, sodium fluoride, and potassium cyanide; he also saw numerous rod-shaped bacteria in stained smears of the organs. The electron micrographs of the present study confirm the microscopic finding of bacteria by Harvey (*3*) and Bassot (*5*). The fact that the light organs contained many tube-like structures and a rich blood supply was interpreted by Harvey (*3*) to mean that these organs were especially constructed to provide the bacteria with nutrients and the large amount of oxygen normally required for luminescence. Moreover, since bacterial luminescence is due to a reaction involving bacterial luciferase, a long-chain aldehyde, reduced FMN, and oxygen (*7*), our finding of light production in cell-free extracts indicates that bacterial luciferase is present in the organs.

Our failure to obtain cultures of luminous bacteria on synthetic agar media agrees with the results of Harvey (*3*) and Haneda (*4*). The failure is very surprising in view of the ease with which luminous bacteria may be cultured from the light organs of practically all luminous fish in which light production has been attributed to bacteria; such fish include *Monocentris, Cleidopus, Physiculus, Coelorhynchus, Paratrachichthys, Acropoma*, and some 20 species of *Leiognathus*. Recently, we have discovered luminous bacteria that cannot be cultured in the light organs of *Leiognathus elongatus* and *Siphamia roseigaster*. It is interesting to note that many insects possess specific symbiotic bacteria which cannot be cultured (*8*). These organisms are believed to add something of value to the economy of the insect, such as to metabolism or nutrition. According to Richards and Brooks (*9*), such symbiotic bacteria called bacteroids live in the oocytes of cockroaches. In this respect, the electron micrographs of Bush and Chapman (*10*) of bacteroids in the developing oocytes of the American cockroach, *Periplaneta americana*, are significant. The morphology of these organisms resembles very strongly the morphology of the organisms shown in Fig. 2 in that each possesses a thin (~ 100 Å) cell wall and a typical unit plasma membrane, as well as nuclear material which lacks a nuclear envelope. In short, each has the characteristics of a prokaryotic cell. In view of these striking resemblances, we suggest that the light organs of *Photoblepharon* and *Anomalops* contain luminous bacteroids that produce the light.

As in nearly all luminous organisms, the function of bioluminescence in *Photoblepharon* and *Anomalops* is unknown. Possibly, the light is used to attract prey or to aid navigation in the dark. The fishermen of Banda, as they did 50 years ago at the time of Harvey's visit, use the light organ as a lure. The organ, cut out of the fish and attached to the line about 10 cm above the baited hook, remains luminous for many hours and is said to attract fish.

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11. This study was carried out during participation by Y.H. and F.I.T. in Program C, *Alpha Helix* 1969 Biological Expedition to New Guinea, Scripps Institution of Oceanography, University of California. It was supported in part by NSF grant GF-274 and the Japan Society for the Promotion of Science under the United States–Japan Cooperative Science Program. We thank the Japan Electron Optics Laboratory for assistance during the electron microscopic work, and Dr. George B. Chapman for helpful comments on the electron micrographs. Dr. John B. Buck, chief scientist of Program C, helped in many ways during the Expedition to make this study possible. This is publication No. 175 of the Department of Biophysics and Microbiology, University of Pittsburgh.

11 February 1971; revised 20 April 1971