Current Comments

William D. McElroy and the Illuminating Story of Bioluminescence

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On a midsummer's evening in the US, anyone gazing at the countryside will notice the twinkling, greenish-yellow lights of the nocturnal beetles known as fireflies, or lightning bugs.[1] Such is the enchantment the insects hold for youngsters that they scamper about in their backyards with empty jars trying to capture the little lights sparkling in the air.

However, more than simple fascination spurs children to such efforts in Baltimore, Maryland. In a tradition begun shortly after World War II by William D. McElroy, then an assistant professor of biology, Johns Hopkins University, local schoolchildren have been enlisted each year to collect fireflies for study by university researchers. "The children are paid a penny for each firefly they bring in," McElroy said recently, "and I've been criticized for over 30 years by people who are afraid we're depleting the firefly population."[2] McElroy is no longer at Johns Hopkins. But the tradition he started is continued by one of his former students, Howard Seliger, who is now a professor of biology at the university.

Due to the notoriety these collection efforts once generated in the local papers, McElroy was one of the first local scientists of whom I learned when I arrived at Johns Hopkins in 1951. By then he was a full professor in the biology department, as well as the director of the McCollum-Pratt Institute, also at Johns Hopkins. McElroy became chairman of the biology department by 1956, a position he held until 1969, when he was appointed director of the National Science Foundation (NSF). After three years at NSF, he was named chancellor of the University of California at San Diego, a position he held until 1980. Currently, he is working as a professor of biology at the university.

In 1964, McElroy was awarded the prestigious Rumford Prize, which is administered by the American Academy of Arts and Sciences in recognition of important discoveries concerning heat or light. Among the numerous professional societies of which McElroy is a member, he is a former president of the American Society of Biological Chemists, the American Institute of Biological Sciences, and the American Association for the Advancement of Science (AAAS). He was a member of the President's Science Advisory Committee under both John F. Kennedy and Lyndon B. Johnson. He was elected to the US National Academy of Sciences (NAS) in 1974, and has served on its council. He was chairman of the board of directors of AAAS from 1977 through 1978. McElroy is currently a member of the NAS's Panel of Reviewers. Particularly relevant to the theme of this essay, he is also the president of the International Congress of Photobiology.

McElroy and I are both members of the board of directors of *Annual Reviews*. In fact, it was during a meeting of the board of *Annual Reviews* that I realized that I had never written anything about his distinguished career, or the subject to which he has contributed so much: bioluminescence.

McElroy has published over 100 journal articles, and edited or coedited numerous books and conference proceed-

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ings. He has been cited in over 3,000 references to articles in which he was the primary author. And this, of course, is based just on the coverage of *Science Citation Index*® (*SCI*®) since 1961. This is remarkable, considering that virtually all his research was completed much earlier. This includes two of his most important discoveries. We'll know more about this when we complete *SCI* for the years 1955-1960 later this year. To comprehend the significance of McElroy's work, however, it is first necessary to understand some of the basic concepts of bioluminescence.

Light is the result of vibrating electric and magnetic fields. That portion of the electromagnetic spectrum to which the naked eye is sensitive is termed "visible light." A special name, however, is applied to any light given off by chemical reactions within a living organism: bioluminescence. Bioluminescence has sparked the curiosity of scientist and layperson alike for thousands of years. It was first described by Aristotle (384-322 BC),[3] whose work was enlarged upon in the first century AD by the Roman scholar Pliny the Elder (23-79 AD).[4]

It is more difficult to pinpoint precisely when the first "modern" study of bioluminescence was conducted. However, one of the earliest scientific descriptions of the phenomenon was made by British scientist Robert Boyle (1627-1691). Boyle's law is known to every chemist, and relates the inverse relationship of pressure and volume of a gas at constant temperature. However, he also helped establish some of the most basic properties of bioluminescent systems. It was Boyle who discovered that luminescence is generated virtually without heat. He also found that the intensity of the light depends on the amount of air (oxygen) present.[4] However, J. Woodland Hastings, Harvard University, Cambridge, Massachusetts, who did his postdoctoral work under McElroy, believes that the term "bioluminescence" first came into general use late in the nineteenth century.[5] And according to E. Newton Harvey in *A History of Luminescence*, the first to use the term "luminescence" was German physicist and historian of science Eilhard Wiedemann (1852-1928) in 1888.[6]

Early observations of bioluminescence—most predating Boyle—generally were not up to the standards of scientific precision that we take for granted today.[6] For instance, early naturalists tended to attribute luminescence to any unusual body structure or marking exhibited by the creatures they were studying.[3] Yet no attempts were made to empirically verify the claims. Some studies proclaimed whole phyla of animals luminescent, based on the evidence of a single specimen. Oftentimes, the specimen turned out to be a nonluminous organism infested by luminous bacteria or fungi. Indeed, it was not until the mid-nineteenth century that the role of luminous bacteria was recognized by Austrian physiologist Johann Florian Heller (1813-1871).[3] These bacteria were responsible for the eerie glow emitted by certain specimens of dead fish. Prior to Heller's discovery, it had been believed that the glow was some property inherent in the fish themselves. And not until the late-nineteenth century was it discovered that symbiotic bacteria were the source of the light exhibited by many

animals thought to be self-luminous, such as many species of deep-sea fish.[3]

Today, we know that a bioluminescent reaction in most cases occurs when organic molecules known generically as luciferins are oxidized in the presence of enzymes called luciferases. These terms were coined by French biologist Raphael Dubois (1849-1929), who was the first to isolate the substances.[3,4] Although details of the chemical reaction differ from organism to organism, generally speaking, luciferin is converted during the process of oxidation from a low-energy, ground-state compound to one in a high-energy, "excited" state. It then loses its energy by radiating a photon of visible light, yielding an oxidized form of luciferin as a by-product. Light is generated until all the luciferin has been oxidized.[7,8] The reaction is remarkably efficient, with virtually no energy wasted as heat—hence the term "cold light."[3,4]

Minor variations on the luciferin/luciferase/oxygen theme do exist. Certain marine worms are luminescent due to a reaction between luciferin, luciferase, and hydrogen peroxide.[3] In a few atypical cases—such as in certain clams, jellyfish, marine worms, and shrimp—a bioluminescent reaction is employed that does not use luciferin at all. Instead, a luminescent protein, or photoprotein, is the substrate, or the substance acted upon, by means of which light is generated. An even more exotic system is employed by a marine dinoflagellate, in which a crystalline-like particle called a scintillon takes part in the emission of a flash of light whenever the acid content of the surrounding water rises above a certain level. These variations in the generation of luminescence, as well as differences in the molecular structures of luciferin and luciferase from species to species, are thought to account for the diversity of color and intensity of light seen among different organisms.[3,4]

Perhaps the two most significant modifications of the basic luciferin/luciferase reaction were identified by McElroy, one before he had even made full professor at Johns Hopkins. In his landmark 1947 paper, entitled "The energy source for bioluminescence in an isolated system," McElroy reported his observation that adding adenosine triphosphate (ATP), a high-energy compound found in all living cells, to samples of ground fireflies caused a brilliant flash of light to appear immediately, and persist for a considerable time, depending upon the concentration of the ATP.[9] Since the publication of McElroy's paper, it has been found that ATP functions with luciferase in catalyzing the reaction between luciferin and oxygen in firefly luminescence.[3,4,10-12] And, in 1953, McElroy, Hastings, and colleagues found that in bacterial luminescence, luciferase catalyzes the oxidation of a reduced form of luciferin, flavin mononucleotide ($FMNH_2$)—a compound highly important in cellular respiration—and a long-chain aldehyde.[13] The compound resulting from the oxidation of the flavin reacts with the aldehyde to form a chemical in an excited state, which releases its energy in the form of light plus a number of by-products.[14-25]

Virtually every application of bioluminescence in industry and research laboratories derives its usefulness from the fact that ATP and $FMNH_2$ are the limiting factors for the amount and intensity of light radiated from a sample. Thus, each application owes at least some debt to the discoveries of McElroy and Hastings. I anxiously await the preparation of *SCI* for the postwar period so we can trace and map the impact of these discoveries.

McElroy first became interested in bioluminescence as a graduate student at Stanford University's Hopkins Marine Station at Monterey, when he took a course under microbiologist C.B. Van Niel. His interest was further stimulated when he went to Princeton University to finish work on his doctorate. There he met Harvey, an acknowledged giant in biochemistry and bioluminescence, who was to become McElroy's mentor. "I had become interested in bioluminescence from an energetic standpoint," McElroy says, "because it is a most unusual biochemical reaction. Whereas in

biochemistry, most steps take place in small, discrete energy units, things were obviously different in bioluminescence. It's a sort of reverse photosynthesis in big quantum jumps."[2]

McElroy's breakthrough concerning the nature of firefly luminescence has had profound effects on the direction of bioluminescence research. But McElroy insists he was merely in the right field at the right time. "Dubois's experiments were the beginning of the biochemistry of bioluminescence, really," he notes. "But I think it's fair to say that it wasn't until after World War II that the field of biochemistry began to mushroom. I just happened to be there at the right time. Repeating Dubois's old experiments is all we were doing, to see what he had found—and it turned out he had found ATP, and hadn't even known it."[2]

The field of bioluminescence has greatly expanded since 1947, when McElroy first identified ATP as the limiting agent in firefly luminescence. And ISI®'s data bases reflect that expansion. Table 1 presents a selected bibliography of the articles retrieved by simply using research front #79-1255, entitled "Bacterial bioluminescence," from our *ISI/BIOMED*™ online data base.[26] Table 2 provides a selected list of the articles retrieved from research front #80-1983, entitled "Biochemistry of bacterial bioluminescence." The core papers for each of these research fronts are shown in Tables 3 and 4. I've also included a cluster map in Figure 1, to illustrate the co-citation relationships among the core papers for "Bacterial bioluminescence."

It is noteworthy that Hastings's papers are featured prominently in the map. The impact of McElroy's work on the field is still felt at key institutions across the US, where bioluminescence re-

Table 1: A selected bibliography of papers retrieved from *ISI/BIOMED*™ online data base by using research front #79-1255, "Bacterial bioluminescence."

Baldwin T O, Ziegler M M & Powers D A. Covalent structure of subunits of bacterial luciferase: NH_2-terminal sequence demonstrates subunit homology. *Proc. Nat. Acad. Sci. US—Biol. Sci.* 76:4887-9, 1979.

Baumstark A L, Cline T W & Hastings J W. Reversible steps in the reaction of aldehydes with bacterial luciferase intermediates. *Arch. Biochem. Biophys.* 193:449-55, 1979.

Eberhard A, Hinton J P & Zuck R M. Luminous bacteria synthesize luciferase anaerobically. *Arch. Microbiol.* 121:277-82, 1979.

Ghisla S, Hastings J W, Favaudon V & Lhoste J M. Structure of the oxygen adduct intermediate in the bacterial luciferase reaction: ^{13}C nuclear magnetic resonance determination. *Proc. Nat. Acad. Sci. US* 75:5860-3, 1978.

Hart R C & Cormier M J. Recent advances in the mechanisms of bio- and chemiluminescent reactions. *Photochem. Photobiol.* 29:209-15, 1979.

Hastings J W, Tu S C, Becvar J E & Presswood R P. Bioluminescence from the reaction of FMN, H_2O_2 and long chain aldehyde with bacterial luciferase. *Photochem. Photobiol.* 29:383-7, 1979.

Makemson J & Hastings J W. Inhibition of bacterial bioluminescence by pargyline. *Arch. Biochem. Biophys.* 196:396-402, 1979.

Makemson J C & Hastings J W. Poising of the arginine pool and control of bioluminescence in *Beneckea-harveyi*. *J. Bacteriol.* 140:532-42, 1979.

Presswood R P & Hastings J W. Steps in the population of the emitter in the bacterial luciferase reaction. *Photochem. Photobiol.* 30:93-9, 1979.

Tu S C. Isolation and properties of bacterial luciferase-oxygenated flavin intermediate complexed with long-chain alcohols. *Biochemistry—USA* 18:5940-5, 1979.

Ulitzur S. A sensitive bioassay for lipase using bacterial bioluminescence. *Biochim. Biophys. Acta* 572:211-7, 1979.

Ulitzur S & Hastings J W. Control of aldehyde synthesis in the luminous bacterium *Beneckea-harveyi*. *J. Bacteriol.* 137:854-9, 1979.

Ulitzur S & Hastings J W. Evidence for tetradecanal as the natural aldehyde in bacterial bioluminescence. *Proc. Nat. Acad. Sci. US—Biol. Sci.* 76:265-7, 1979.

Ulitzur S & Heller M. A new, fast, and very sensitive bioluminescence assay for phospholipase-A and C. *Anal. Biochem.* 91:421-31, 1978.

Whitehead T P, Kricka L J, Carter T J N & Thorpe G H G. Analytical luminescence: its potential in the clinical laboratory. *Clin. Chem.* 25:1531-46, 1979.

Table 2: A selected bibliography of papers retrieved from *ISI/BIOMED*™ online data base by using research front #80-1983, "Biochemistry of bacterial bioluminescence."

Barak M & Ulitzur S. Bacterial bioluminescence as an early indication of marine fish spoilage. *Eur. J. Appl. Microbiol. Biotech.* 10:155-65, 1980.

Baumann P, Baumann L, Bang S S & Woolkalis M J. Reevaluation of the taxonomy of *Vibrio, Beneckea*, and *Photobacterium*: abolition of the genus *Beneckea*. *Curr. Microbiol.* 4:127-32, 1980.

Haas E. Bioluminescence from single bacterial cells exhibits no oscillation. *Biophys. J.* 31:301-12, 1980.

Holländer R & Pohl S. Deoxyribonucleic acid base composition of bacteria. *Zbl. Bakt. Mikrobiol. Hyg. A—Med.* 246:236-75, 1980.

Holzman T F & Baldwin T O. Proteolytic inactivation of luciferases from three species of luminous marine bacteria, *Beneckea-harveyi, Photobacterium-fischeri*, and *Photobacterium-phosphoreum*: evidence of a conserved structural feature. *Proc. Nat. Acad. Sci. US—Biol. Sci.* 77:6363-7, 1980.

Jensen M J, Tebo B M, Baumann P, Mandel M & Nealson K H. Characterization of *Alteromonas-hanedai* (sp. nov.), a nonfermentative luminous species of marine origin. *Curr. Microbiol.* 3:311-5, 1980.

Karl D M & Nealson K H. Regulation of cellular metabolism during synthesis and expression of the luminous system in *Beneckea* and *Photobacterium*. *J. Gen. Microbiol.* 117:357-68, 1980.

Meighen E A & Bartlet I. Complementation of subunits from different bacterial luciferases. *J. Biol. Chem.* 255:11181-7, 1980.

Merritt M V & Baldwin T O. Modification of the reactive sulfhydryl of bacterial luciferase with spin-labeled maleimides. *Arch. Biochem. Biophys.* 202:499-506, 1980.

Nealson K H & Hastings J W. Bacterial bioluminescence: its control and ecological significance. *Microbiol. Rev.* 43:496-518, 1979.

Orndorff S A & Colwell R R. Distribution and identification of luminous bacteria from the Sargasso Sea. *Appl. Environ. Microbiol.* 39:983-7, 1980.

Ruby E G, Greenberg E P & Hastings J W. Planktonic marine luminous bacteria: species distribution in the water column. *Appl. Environ. Microbiol.* 39:302-6, 1980.

Ruby E G & Hastings J W. Formation of hybrid luciferases from subunits of different species of *Photobacterium*. *Biochemistry—USA* 19:4989-93, 1980.

Ruby E G & Hastings J W. Proteolytic sensitivity of the α subunit in luciferases of *Photobacterium* species. *Curr. Microbiol.* 3:157-9, 1979.

Tebo B M, Linthicum D S & Nealson K H. Luminous bacteria and light emitting fish: ultrastructure of the symbiosis. *BioSystems* 11:269-80, 1979.

Tu S C & Hastings J W. Physical interaction and activity coupling between two enzymes induced by immobilization of one. *Proc. Nat. Acad. Sci. US—Biol. Sci.* 77:249-52, 1980.

Ulitzur S & Hastings J W. Reversible inhibition of bacterial bioluminescence by long-chain fatty acids. *Curr. Microbiol.* 3:295-300, 1980.

Ulitzur S, Weiser I & Yannai S. A new, sensitive and simple bioluminescence test for mutagenic compounds. *Mutat. Res.* 74:113-24, 1980.

search is being advanced by others who, like Hastings, did their postdoctoral work with McElroy, as well as several of McElroy's former students. The list includes: M. Cormier, University of Georgia, Athens; Seliger; and McElroy's wife, Marlene DeLuca, University of California at San Diego, La Jolla. Through these workers, McElroy's influence on the field is truly vast. For instance, all of the authors except one in Table 1 have either done their postdoctoral work with Hastings or presently work in his lab. "I guess much of the expansion in the field of bioluminescence was just a matter of infiltrating the academic community with my products,"[2] McElroy says with a chuckle.

The bioluminescent organisms that are the focus of all this research activity are found not only among bacteria and fungi, but also among unicellular algae and most of the major animal phyla—some of which contain hundreds of luminescent species.[12] A partial list includes not only the creatures familiarly known as fireflies, glowworms, automobile bugs, cucujos, star worms, and railroad worms, but also various protozoa, corals, marine worms, and earthworms; clams, snails, and squid, as well as three species of octopus; sea spiders, millipedes, centipedes, various crustaceans, and insects; and starfish, brittle stars, and numerous bony fish.[3,27]

It seems reasonable to assume that bioluminescence must serve some important purpose in the organisms that have developed it, since the energy required to emit light is significant.[4,7,8]

Table 3: Core papers for the research front #79-1255, entitled "Bacterial bioluminescence," retrieved from *ISI/BIOMED*™ online data base.

Entsch B, Ballou D P & Massey V. Flavin-oxygen derivatives involved in hydroxylation by *p*-hydroxybenzoate hydroxylase. *J. Biol. Chem.* 251:2550-63, 1976.
Hastings J W, Balny C, Le Peuch C & Douzou P. Spectral properties of an oxygenated luciferase-flavin intermediate isolated by low-temperature chromatography. *Proc. Nat. Acad. Sci. US* 70:3468-72, 1973.
Hastings J W & Nealson K H. Bacterial bioluminescence. *Annu. Rev. Microbiol.* 31:549-95, 1977.
Hastings J W & Weber G. Total quantum flux of isotropic sources. *J. Opt. Soc. Amer.* 53:1410-5, 1963.
Hastings J W, Weber K, Friedland J, Eberhard A, Mitchell G W & Gunsalus A. Structurally distinct bacterial luciferases. *Biochemistry—USA* 8:4681-9, 1969.
Kemal C & Bruice T C. Simple synthesis of a 4a-hydroperoxy adduct of a 1,5-dihydroflavine: preliminary studies of a model for bacterial luciferase. *Proc. Nat. Acad. Sci. US* 73:995-9, 1976.
Mitchell G W & Hastings J W. A stable, inexpensive, solid-state photomultiplier photometer. *Anal. Biochem.* 39:243-50, 1971.
Reichelt J L & Baumann P. Taxonomy of the marine, luminous bacteria. *Arch. Mikrobiol.* 94:283-330, 1973.

Table 4: Core papers for research front #80-1983, entitled "Biochemistry of bacterial bioluminescence," retrieved from *ISI/BIOMED*™ online data base.

Hastings J W & Nealson K H. Bacterial bioluminescence. *Annu. Rev. Microbiol.* 31:549-95, 1977.
Mitchell G W & Hastings J W. A stable, inexpensive, solid-state photomultiplier photometer. *Anal. Biochem.* 39:243-50, 1971.
Reichelt J L, Baumann P & Baumann L. Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium* by means of in vitro DNA/DNA hybridization. *Arch. Microbiol.* 110:101-20, 1976.
Reichelt J L & Baumann P. Taxonomy of the marine, luminous bacteria. *Arch. Mikrobiol.* 94:283-330, 1973.

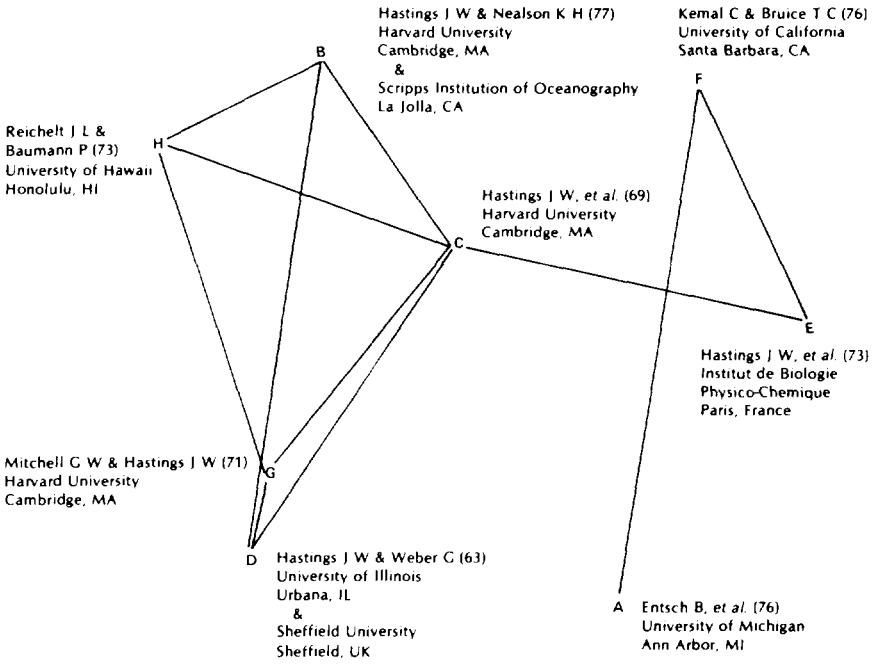
Yet during the prescientific era, it was considered merely a functionless curiosity.[3,4,6] After all, of what possible use could bioluminescence be to a bacterium? Bioluminescence was commonly regarded as the whimsical action of a bemused Creator, providing humans with a spectacle that delighted the eye and challenged the mind.[3,4,6]

Yet, bioluminescence is indeed essential to the protection and survival of the species associated with it.[3,4,10,14,28] In many organisms, luminescence appears most intensely after they have been frightened or irritated. For example, turbulent water—such as might be churned up by marauding fish—will cause luminescent plankton, dinoflagellates, and other unicellular organisms to glow brilliantly *en masse*. In turn, the very water itself appears luminous—possibly serving to frighten the predator away. Some deep-sea squid and octopuses, when frightened, release a cloud of luminous secretion to confuse predators and mask an escape.[3,4,10,14,28]

Various organisms, including the firefly, use bioluminescence as a species- or sex-recognition pattern.[3,4,10,14,28] Deep-sea fish and other marine animals may use bioluminescence to attract prey, to disguise their silhouette, or simply to light their way through an environment never penetrated by natural light. Even bacterial luminescence is thought to serve a purpose.[14,29] Since the glow of luminous bacteria is oxygen-dependent, the ability to emit light may have developed as a method of removing oxygen from Earth's primordial atmosphere.[14,29] Oxygen is a deadly poison to many primitive organisms. However, most researchers feel that the emitted light is of functional importance, and that its perception by other organisms—such as fish and other predators—ultimately serves to the advantage of the luminous bacteria.[14] For example, the light may attract a predator and encourage it to consume the bacteria—following which the bacteria take up symbiotic residence in the predator's gut. Or the light may serve to frighten away the predator.[14]

Among the most complex adaptations of bioluminescence to be found in na-

Figure 1: Cluster map showing co-citation relationships among the core papers from *ISI/BIOMED*™ research front #79-1255, "Bacterial bioluminescence."



ture are those exhibited by fireflies, which are among the most intensely studied of all luminescent organisms. The flickerings of fireflies on a meadow in summer may inspire a feeling of pastoral contentment in the casual observer. The reality behind the twinkling facade, however, is one of competition, deception, and predation.[30] Within 24 hours of a firefly's emergence as an adult, it is capable of producing the intricately coordinated flashes characteristic of its particular species.[31] In North America, the males of various species take to the air to advertise their availability for mating, while the females remain grounded. In a given area, there may be any number of species intermingling, both in the air and on the ground, and males may outnumber females by as much as 50 to one.[30]

From the male firefly's point of view, mating is a task that cannot be taken lightly. He must fly about at the proper altitude, at the proper speed, at the proper time of the evening during the proper time of year, and emit light in the appropriate manner, merely to succeed in attracting the attention of a female of his own species. He must then continue flashing his light and approach her in the correct way, or she will stop responding to him and he will lose her. If he is slow to answer a female response, or bungles his reply, he is likely to lose out to a rival male. Yet he cannot answer too eagerly, for once the females of certain species have mated, they no longer give their own response pattern. Instead, they mimic the pattern of females of other species, luring unsuspecting males to their sides and then devouring them.[30]

Factors beyond the mere complexity of the luminescent signal/response pattern may complicate matters still further for the male. For instance, many species of firefly are sexually active for only a very limited period each night, some-

times as little as 15 or 20 minutes. A crash landing by a hapless male, should he survive it, may cost him up to 15 precious seconds. Should a male mistakenly answer the response signal of a female of another species, he may waste much of the evening before he discovers his blunder. And males of certain species, frustrated after many evenings of fruitless courtship, add to the troubles of their comrades who have not yet given up by landing and flashing various female responses. Females themselves, incidentally, have little trouble mating. Most are able to attract a male, entice him to the ground, copulate for 90 seconds, and return to their burrow, all in a matter of minutes.[30]

Fireflies in the region extending east and southwest from India to the Philippines and New Guinea have evolved a pattern of flashing behavior far different than that of their North American cousins. In areas stretching for miles along the rivers and swamps of Asia and the Pacific islands, several species of fireflies gather in trees each night, swarming by the thousands, males as well as females, each species to its own tree. In perhaps one of the most breathtaking spectacles in nature, each dense swarm begins to flash on and off, in perfect unison. The insects take time out from their flashing duties only to mate.[32,33]

In recent years, investigators have begun applying their knowledge of bioluminescence toward accomplishing human tasks. Bioluminescence forms the basis of numerous procedures in biology, immunology, biochemistry, and medicine.[34-37] Its usefulness is based primarily on the dependence of bacterial luminescence on $FMNH_2$, and of firefly luminescence upon ATP.[37] The amount of light emitted during the course of most testing procedures is directly proportional to the amount of $FMNH_2$ or ATP present in the sample.[38] Thus, in the case of firefly luminescence, preparations of purified luciferin and luciferase, or crude extracts of the firefly's light-generating organ, or lantern—and even the dried lantern itself—can be used to test for the presence of ATP, and for monitoring ATP-converting reactions.[39]

Due to the ease with which luminescent bacteria can be cultured, as well as the simple procedures involved in making use of firefly luminescence, the possibilities for applications of bioluminescence research are many. Firefly luminescence, for example, may be used to test for the presence of bacteria in biological fluids by measuring the concentration of ATP of bacterial origin. The method is useful for quantifying the bacteria in blood,[39] and has more exotic applications as well. It can, for example, determine the types of bacteria present in dental plaque, a tarry substance implicated in the formation of cavities.[40] To study the etiology of plaque-caused cavity formation, clinicians must first identify plaque's bacterial components. Bioluminescence enables researchers to identify and quantify bacteria by their patterns of ATP production.[40]

Incidentally, this method should not be confused with the type of procedure your dentist may use—as does mine. My dentist, Lew Abrams, and his assistant, hygienist Anne Marie MacFadden, use a liquid called *Plaque-Check*, manufactured by Bristol Meyers, to determine the pattern of plaque formation on teeth. *Plaque-Check*'s active ingredient is a fluorescing sodium compound. When applied to teeth, it adheres to the areas where plaque is present. By using a mirror and an ultraviolet light, a patient can see the places where he or she needs to brush harder. This is an ingenious application of fluorescence, however, not bioluminescence.

As I mentioned earlier, bioluminescence makes it possible to investigate the growth and physiology of various microorganisms that have proved difficult to study with other techniques. The presence and amount of compounds that influence bacterial growth, such as vitamins, can be determined by using bioluminescence to quantify increases in the rate of ATP production (and, by implication, increases in the rate of

bacterial growth). Conversely, the concentration of an antibiotic or the susceptibility of bacteria to an antibiotic may be determined by using bioluminescence to quantify decreases in the rate of ATP production.[39]

Perhaps one of the most important applications yet found for bioluminescence is in the detection of cancer. Many kinds of tumors release a number of by-products of their growth into the bloodstream of the victim. One of these products is called *creatine kinase isoenzyme BB*.[41] Creatine kinase is capable of taking part in reactions by which ATP is formed or consumed. So its concentration can be determined through the use of bioluminescent procedures. In fact, the rate of ATP production in a sample is directly proportional to the amount of creatine kinase present. From this, the presence and severity of the disease may be inferred.[39,41]

Another important laboratory application of bioluminescence is as a replacement for the radioimmunoassay.[42,43] In the radioimmunoassay, a radioactive compound is used as a "tracer" or "marker," to identify some specific substance for study. The radioactivity of the tracer allows the investigator to keep track of the substance as it takes part in the body's metabolic processes. Luminescent assays may make use of either firefly luminescence or bacterial luminescence, and can be employed to determine the nature of a vast number of ATP- and $FMNH_2$-converting processes.[42,43] Not only are luminescent assays more specific and sensitive than radioimmunoassays, they are far less expensive, simpler to use, and yield results more rapidly. Moreover, they do not expose laboratory personnel to the hazards of radioactivity, as do radioimmunoassays. Unfortunately, although the bioluminescent assay has enormous potential, it is still a procedure to be found primarily in the research lab, rather than a commercial technique available for routine clinical use. McElroy and DeLuca have reviewed this in a Japanese journal.[42]

In industry, the principles of the bioluminescent reaction—in combination with a device known as the photomultiplying monitor—are used in various quality-control procedures. A photomultiplier is a machine that is supersensitive to light. Hooked into a computer, it can quantify the minute amounts of light given off by most organic compounds as they slowly decay from contact with oxygen in the air. Photomultipliers are even capable of distinguishing between different elements on the basis of the particular wavelength of light each gives off. Thus, samples of gasoline and motor oil taken during the refining process may be subjected to luminescent tests designed to detect the amount of nitrogen present. A high nitrogen concentration indicates a low-grade or poor-quality product. In fact, the use of luminescent testing procedures ranges from aiding in the regulation of automobile air pollution and making better-quality plastic wraps to measuring the rate at which various foods spoil and the rate at which auto tires wear out. Luminescence can even aid in describing and studying the respiration patterns of farm crops.[44]

Bioluminescence continues to fascinate scientists throughout the world. Who would have believed that from McElroy's curiosity there would have emerged the many practical applications I have cataloged? But my inventory has omitted the ultimate practical benefit of research on the biological conversion of chemical energy into light: the *Cyalume* lightstick, manufactured by American Cyanamid. The only commercial application of luminescence directly available to the consumer, the *Cyalume* lightstick is a clear, wand-shaped plastic tube containing two liquids held apart from one another by a glass vial. When the tube is flexed, the vial breaks, allowing the chemicals to mix and react. Based on the principles of the firefly's luciferin/luciferase reaction, the *Cyalume* produces a cold, yellow-green light that is bright enough to read by for three hours, and remains visible for nine more. At 16

percent efficiency, however, it doesn't even approach the miraculous economy (88 percent efficiency) of the firefly luminescence it mimics.[44]

In spite of the increasing usefulness of bioluminescence to humanity—or perhaps because of that usefulness—we may be taking luminescence for granted. Perhaps, for humans, the importance of bioluminescence does not lie in its practical applications, or in scholarly articles or research fronts. None of these properly reflects the curious fascination that the eerie red, green, yellow, and blue lights have held for humanity since the dawn of history. Perhaps the true value of bioluminescence lies in the delighted faces of children, as they laugh and point at the twinkling lights hovering in the summer dusk. When humanity begins to occupy outer space, will we take the firefly along to remind us of what life once was like on earth?

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