

# Bacterial Bioluminescence: Its Control and Ecological Significance

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<b>INTRODUCTION</b> .....	<b>496</b>
<b>BIOCHEMISTRY</b> .....	<b>497</b>
<b>TAXONOMIC RELATIONSHIPS OF LUMINOUS BACTERIA</b> .....	<b>499</b>
<b>Marine Forms</b> .....	<b>499</b>
<b>Terrestrial and Freshwater Forms</b> .....	<b>501</b>
<b>CONTROL OF THE SYNTHESIS AND ACTIVITY OF THE LUMINESCENT SYSTEM</b> .....	<b>501</b>
<b>Autoinduction</b> .....	<b>501</b>
<b>Catabolite Repression</b> .....	<b>503</b>
<b>Composition of the Growth Medium</b> .....	<b>503</b>
<b>Oxygen</b> .....	<b>504</b>
<b>Pyruvate and Excretion Products</b> .....	<b>504</b>
<b>Dark (K) Variants of Luminous Bacteria</b> .....	<b>504</b>
<b>HABITATS AND DISTRIBUTION OF LUMINOUS BACTERIA</b> .....	<b>505</b>
<b>Free Living</b> .....	<b>505</b>
<b>Saprophytic Forms: Natural Enrichments</b> .....	<b>507</b>
<b>Commensal Forms</b> .....	<b>508</b>
<b>Parasites</b> .....	<b>508</b>
<b>Marine</b> .....	<b>508</b>
<b>Nonmarine</b> .....	<b>508</b>
<b>Light Organ Symbionts</b> .....	<b>509</b>
<b>Teleost fishes</b> .....	<b>509</b>
<b>Squid</b> .....	<b>510</b>
<b>Nonculturable symbionts</b> .....	<b>510</b>
<b>Endosymbionts</b> .....	<b>512</b>
<b>FUNCTIONS OF BIOLUMINESCENCE</b> .....	<b>512</b>
<b>Vestigial or Nonfunctional Hypotheses</b> .....	<b>512</b>
<b>Biochemical Functions</b> .....	<b>513</b>
<b>Biological Functions</b> .....	<b>513</b>
<b>Light organ luminescence</b> .....	<b>513</b>
<b>Dispersion and propagation</b> .....	<b>513</b>
<b>PERSPECTIVES: LUMINOUS AND NONLUMINOUS BACTERIA</b> .....	<b>514</b>
<b>LITERATURE CITED</b> .....	<b>515</b>

## INTRODUCTION

Ten years ago there existed very little data relevant to the ecology of the bioluminescent bacteria and the functional importance of light emission. Although this lack of data did not dampen enthusiasm for speculation as to the function(s) and selective advantage of light emission in bacteria, it did make the formulation of hypotheses difficult. Over the past decade, however, a considerable amount of new knowledge has accumulated, the synthesis and analysis of which provide a foundation for new perspectives and postulates.

The results of taxonomic studies that were directed primarily at problems of systematics have ecological implications and have provided the tools for extensive ecological and distribu-

tional studies. It is now clear that light organ symbioses represent specific advantageous associations between luminous bacteria and fish and that some species of luminous bacteria are not found as light organ symbionts. This has raised the question of whether the light from bacteria that are not light organ symbionts might have adaptive functions, such as enhancing the dispersion and propagation of these bacteria.

Likewise, the results of investigations into the mechanisms controlling bioluminescence that were directed at physiological questions have ecological implications, the most important of which is that these bacteria may be luminous under some conditions and nonluminous under others; i.e., the system is inducible and repressible.

These perspectives and other specific postulates presented here concerning the nature and functions of bacterial bioluminescence provide new approaches for data collection and experimental work.

### BIOCHEMISTRY

The biochemistry of bacterial bioluminescence has been reviewed recently (40); the short summary below serves to introduce the subject and to provide a perspective for the subsequent sections.

The light-emitting reaction of luminous bacteria involves a luciferase-catalyzed oxidation of reduced flavin mononucleotide (FMNH<sub>2</sub>) by molecular oxygen, with the concomitant oxidation of a long-chain aliphatic aldehyde, probably tetradecanal (93). Although the biochemical system is unique to bacteria, it is nevertheless found in higher organisms by virtue of the occurrence of luminous bacteria in mutualisms of several types (see below). Recent work has also shown that bacterial luciferase is in fact not confined to the bioluminescent species of bacteria; many closely related nonluminous species contain luciferase in low but readily detectable amounts (K. H. Neelson and D. S. Walton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I131, p. 102).

Luciferase can be viewed as a mixed-function oxidase, involving the oxidations of FMNH<sub>2</sub> and a long-chain aliphatic aldehyde in a reaction that is analogous to the reactions of certain flavoprotein hydroxylases (37). An unusual feature of the reaction is its inherent slowness; at room temperature the time required for a single luciferase cycle to occur is on the order of 10 s (38), making it one of the slowest enzymes known. A practical consequence of this is that there are intermediates in the reaction with long lifetimes. Indeed, by using low temperatures, these have been isolated, purified, and characterized (2, 9, 37a); the initial chemical species is postulated to be a luciferase-bound 4a-peroxy adduct of FMNH<sub>2</sub> (23).

Luciferases from several different luminous bacteria have been isolated, purified, and studied; these bacteria include five marine species (*Beneckea* [*Vibrio*] *harveyi*, *Beneckea splendida*, *Photobacterium fischeri*, *Photobacterium phosphoreum*, and *Photobacterium leiognathi*) and two terrestrial-freshwater species (*Vibrio cholerae* biotype *albensis* and *Xenorhabdus luminescens*) (See below). Although there are significant differences, all bacterial luciferases possess a heterodimeric structure and have molecular weights in the range 76,000 ± 4,000 (40; E. G. Ruby and J. W. Hastings, unpublished data; K. Kopecky and K. H. Neelson, unpub-

lished data). All exhibit high specificity for FMNH<sub>2</sub> and a long-chain aliphatic aldehyde (8 to 16 carbons) (41, 97). For luciferases from different strains of a given species, the kinetics of decay with a given aldehyde are similar (J. M. Fitzgerald, Ph.D. thesis, Monash University, Victoria, Australia, 1979; Neelson, unpublished data); with dodecanal as the aldehyde in the *in vitro* assay, all luciferases from *Photobacterium* isolates so far examined exhibit fast decay, whereas all luciferases from *Beneckea* exhibit slow kinetics (Fig. 1). The turnover rates of the luciferases thus form the basis for rapid distinction among the major bacterial groups (64).

The subunit structure of luciferase, as exemplified by the luciferase of *B. harveyi*, is a heterodimer having a molecular weight of 79,000, whose two nonidentical subunits (alpha, 42,000 daltons; beta, 37,000 daltons) can be isolated in quantity by diethylaminoethyl-Sephadex chromatography in 5 M urea. Individual subunits are inactive but can be recombined to yield a recon-

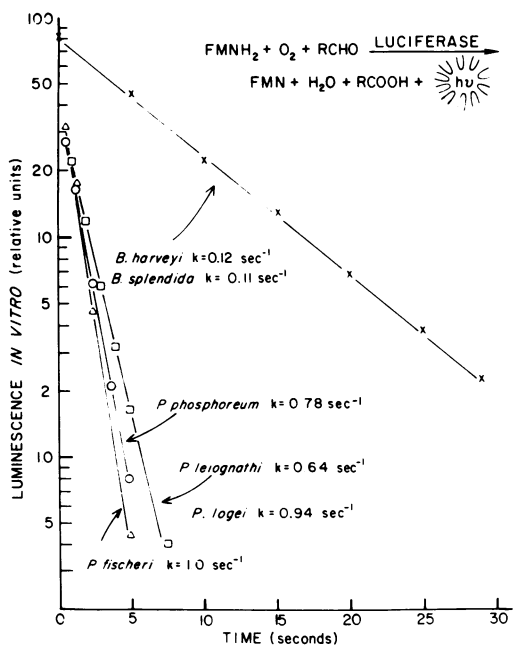


FIG. 1. Luciferase kinetics in the nonturnover assay. The kinetics of the decay of luminescence from the luciferase reaction *in vitro* (the reaction sequence is shown in the upper right) when initiated with FMNH<sub>2</sub> in the presence of dodecanal are shown for luciferases isolated from several species of marine bacteria. The apparent first-order rate constants (*k*) for the decay of luminescence are indicated for two species not graphed. All *Beneckea* species display slow kinetics, whereas all *Photobacterium* species show fast kinetics. RCHO, Long-chain aliphatic aldehyde; FMN, flavin mononucleotide.

stituted luciferase with activity and properties like those of the native material. From mutant analyses (15, 16) and chemical modification studies (60) it is postulated that the catalytic properties reside on the alpha subunit. Although the beta subunit is required for luciferase activity, its specific function has not been elucidated.

Recent determinations of the amino acid sequences of the luciferase subunits of *P. fischeri* and *B. harveyi* indicate clear relatedness between the luciferases of the two genera and also between the two subunits (1). From these data it is possible to postulate that the luciferases in these two species evolved from the same monomer. Complementation studies with subunits from different species, coupled with amino acid sequence data, should lead to insight into the evolution of this protein. For example, active hybrid luciferases have been formed by renaturing the subunits from the three different *Photobacterium* species (Ruby and Hastings, unpublished data). All combinations except *P. fischeri* alpha subunit with *P. phosphoreum* beta subunit give active hybrids. In the hybrids, the properties related to catalytic function (turnover time) are determined by the species contributing the alpha subunit. Similarly, subunits of *B. harveyi* luciferase form active hybrids with those from both *V. cholerae* biotype *albensis* and *X. luminescens* (Kopecky and Nealson, unpublished data). No active hybrids between *Photobacterium* and *Beneckea* luciferase subunits have yet been reported.

The overall reaction of bacterial luciferase results in the production of light. As Fig. 2 shows, the emitter ( $VI^*$ ) is a luciferase-flavin complex, but its specific structure is not yet known. Aldehyde is not required in the light-emitting reaction until the peroxy-FMNH<sub>2</sub> (II) has been formed; in the absence of aldehyde (in vitro) this intermediate (II) breaks down without significant light emission. Mutants in which the synthesis of natural aldehyde is blocked are nonluminous, but normal or even higher levels of active luciferase occur (40). Whether electrons are shunted via this dark pathway in such mutants is not known. In the presence of aldehyde, and presumably in vivo, the light-emitting reaction results in the concomitant formation of the corresponding long-chain fatty acid. Evidence both in vivo (93) and in vitro (59) indicates that this fatty acid is reduced back to the aldehyde (Fig. 2).

In cells the bacterial bioluminescent system can be viewed as a branch (at the level of pyridine nucleotides) of the electron transport pathway, in which electrons from reduced substrates are shunted via reduced flavin to oxygen (Fig.

2). The affinity of the system for oxygen is very high; at very low oxygen concentrations, where cytochrome electron flow may be completely blocked, luminescence can occur without appreciable diminution (32). Under microaerophilic conditions, such a pathway might be advantageous (see below). Since the emission of light requires molecular oxygen, during strict anaerobiosis luminescence ceases, and upon readmission of oxygen an excess flash of luminescence occurs. This can now be interpreted to be the result of the accumulation of luciferase-reduced flavin complex I (L-FH<sub>2</sub> in Fig. 2).

In very bright cells growing in a rich medium, it has been estimated that luciferase accounts for as much as 5% of the cellular protein (40a) and that luminescence accounts for as much as 20% of the total oxygen consumption (32). The luminescence system may thus represent a significant energetic drain. However, in minimal media, when luminescence is much less, the energetic requirements are also much less. It has been estimated, on the basis of energy charge analysis and light measurements (47), that for very bright strains the energy committed to bioluminescence is as much as 10% of the total,

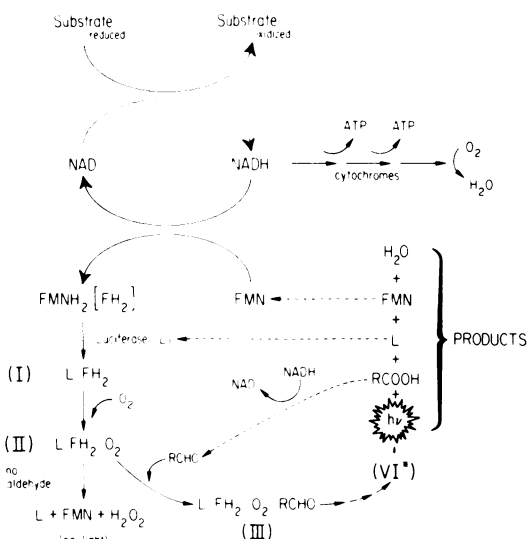


FIG. 2. Pathway of bacterial bioluminescence. The pathway of reactants and proposed intermediates is shown in relation to cellular metabolism and electron flow. For each cycle of light production, one reduced flavin (FMNH<sub>2</sub>) and one reduced pyridine nucleotide (reduced nicotinamide adenine dinucleotide [NADH<sub>2</sub>], used for aldehyde regeneration) are presumably required. NAD, Nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; FMN, flavin mononucleotide; RCHO, long-chain aliphatic aldehyde.

whereas for other bacteria (normal luminous forms) the energetic drain is much less (0.001% or less). Understanding the energetic requirements of the synthesis and activity of the luminous system under different conditions will be required for understanding the relationship between the biochemistry of the system and its ecological significance.

## TAXONOMIC RELATIONSHIPS OF THE LUMINOUS BACTERIA

### Marine Forms

Over the past decade, the taxonomic status of the marine luminous bacteria has been clarified (6-8, 78, 80), allowing for a better appreciation of the key physiological and ecological properties of the different bacterial groups (40). This work has resulted in a wider recognition of the fact that a specific bacterial taxon may have some members that exhibit bioluminescence and others that do not (Table 1).

As shown in Table 1, there are six recognized marine luminous species, all gram-negative motile rods that share a number of morphological, physiological, and biochemical properties with the *Enterobacteriaceae* and the *Vibrionaceae*. They are all facultative anaerobes capable of growth at 25°C on glucose, mannose, galactose,

fructose, glycerol, and *N*-acetylglucosamine, and all produce an extracellular chitinase (90).

The current classification and division into genera are based on (i) the mode of flagellation, (ii) the moles percent guanine plus cytosine content of the DNA, (iii) in vitro deoxyribonucleic acid (DNA)-DNA hybridization and DNA-ribosomal ribonucleic acid hybridization, and (iv) nutritional and enzymatic properties. Based on these criteria, four species are placed in the genus *Photobacterium* and two are placed in *Beneckea*. All luminous *Photobacterium* species, except the newly described *Photobacterium logei*, are known to occur in specific association with a higher organism, whereas no *Beneckea* species have been found as light organ symbionts.

*P. fischeri* cells are yellow pigmented and rod shaped and have a tuft of sheathed flagella (Fig. 3). They are restricted to the marine environment and have a specific requirement for sodium ion for growth (79). They occur as free-living forms and as specific symbionts of monocentrid fishes (21, 86).

*P. logei* is a recently identified species closely related to *P. fischeri* (3). This species is adapted to colder waters, showing an ability to grow at 4°C and an inability to grow at 30°C. In most other aspects it is similar to *P. fischeri*.

TABLE 1. Luminous and nonluminous species of luminous genera

Habitat	Genus	DNA guanine plus cytosine content (mol %)	Luminous species	Nonluminous species		
				Name	Ability to induce luminescence system in <i>B. harveyi</i> <sup>a</sup>	Production of bacterial luciferase <sup>b</sup>
Marine	<i>Photobacterium</i>	39-44	<i>P. fischeri</i> , <i>P. logei</i> , <i>P. phosphoreum</i> , <i>P. leiognathi</i>	<i>P. angustum</i>	-	-
	<i>Beneckea</i> ( <i>Vibrio</i> ) <sup>c</sup>	45-48	<i>B. harveyi</i> , <i>B. splendida</i>	<i>B. alginolytica</i> <i>B. parahaemolytica</i> <i>B. anguillara</i> <i>B. natriegens</i> <i>B. campbelli</i> <i>B. vulnifica</i> <i>B. nigrapulchrituda</i> <i>B. pelagia</i> <i>B. nereida</i>	+ + + + + + - -	+ + + - + - - -
Nonmarine	<i>Vibrio</i>	47-49	<i>V. cholerae</i> biotype <i>albensis</i>	<i>V. cholerae</i>	NT <sup>d</sup>	NT
	<i>Xenorhabdus</i> <sup>e</sup> ( <i>Achromobacter</i> )	43-44	<i>X. luminescens</i> strain Hb (ATCC 29999), strains ATCC 19061, X-II, All, Dn, R	<i>X. nematophilus</i> NC-19 (ATCC 129304) Hb-B, Hm	NT	NT

<sup>a</sup> See reference 25.

<sup>b</sup> See Neilson and Walton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I131, p. 102.

<sup>c</sup> The nomenclature at the generic level for these species is still unsettled. For a discussion of this, see reference 7.

<sup>d</sup> NT, Not tested.

<sup>e</sup> *Achromobacter* is an earlier, now obsolete assignment. See reference 92 for full data on the genus *Xenorhabdus*.

*P. phosphoreum* and *P. leiognathi*, which are taxonomically similar, may also be distinguished by differences in optimal growth temperatures. *P. phosphoreum* is enriched for by incubation at low temperatures (4°C), and its growth is often inhibited by temperatures above 25°C. *P. leiognathi* strains grow well in laboratory culture at temperatures up to 35°C and are commonly found in warm tropical waters. Both species are obligate marine forms with specific requirements for sodium ion. They are short rods with one to three unsheathed polar flagella and, when grown on glucose, possess bright refractile granules of poly-beta-hydroxybutyric acid. Both species also occur as specific light organ symbionts, *P. leiognathi* with members of the family Leiognathidae (82) and *P. phosphoreum* with a variety of midwater and deepwater fishes (45, 84).

The luminous *Beneckeia* species are quite similar and were separated into two taxa only after extensive nutritional analyses (78). *B. harveyi* cells are straight rods, whereas *B. splendida*

cells are more curved; both have one to three sheathed polar flagella. Many but not all strains of *B. harveyi* have sheathed peritrichous flagella that are induced by growth on solid media (Fig. 1). Both species have specific growth requirements for sodium ion. *B. harveyi* occurs in surface and coastal seawaters in relatively high numbers in a seasonal fashion (88; E. G. Ruby, Ph.D. thesis, Scripps Institution of Oceanography, La Jolla, Calif., 1977), whereas the distribution of *B. splendida* has not been documented. Many nonluminous strains that are taxonomically indistinguishable by DNA-DNA hybridization from *B. harveyi* have been isolated from seawater and fish surfaces (8, 78). It is possible that these strains contain very low levels of luciferase, as has been found in some nonluminous species (Nealson and Walton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978), but this question has not been examined.

No *Beneckeia* species have been isolated as specific light organ symbionts, whereas with the exception of *P. logei*, all luminous *Photobacterium* species have been. This raises the possibility that luminous members of the genus *Beneckeia* may be classed as free living and that *Photobacterium* species may be classed as symbionts (39, 40). Among the taxonomic features that distinguish the two genera are large differences in nutritional versatility. The *Beneckeia* strains are more nutritionally versatile, capable of utilizing 28 to 45 (depending on the strain) of 147 different organic compounds as the sole carbon and energy source, whereas the *Photobacterium* strains, including most of those isolated directly from light organs, can utilize only between 7 and 22 of these compounds (78). This suggests that nutritional factors are not involved in strain selection and maintenance in the light organs and that the light organ is not the only niche available for the symbiotic species. The fact that *Beneckeia* isolates are common in surface and coastal waters (and not deeper) (88, 100; Ruby, Ph.D. thesis) may be related to nutritional factors, but much more information is needed. For instance, the capacity of these species to grow under low-nutrient (oligotrophic) conditions has not yet been studied in detail.

Of 150 isolates from the light organ of *Monocentris japonica*, all were *P. fischeri* and grew well on a defined minimal medium without supplements (86). Similarly, all 723 *P. leiognathi* isolates from many leiognathid fishes (82) and 152 *P. phosphoreum* isolates from midwater macrourids and opisthoproctids (84) were prototrophs. On the other hand, naturally occurring auxotrophs of *P. phosphoreum* do occur, as first reported by Doudoroff (19). Reichelt and Baumann (78) found that about 45% of 78 isolates of

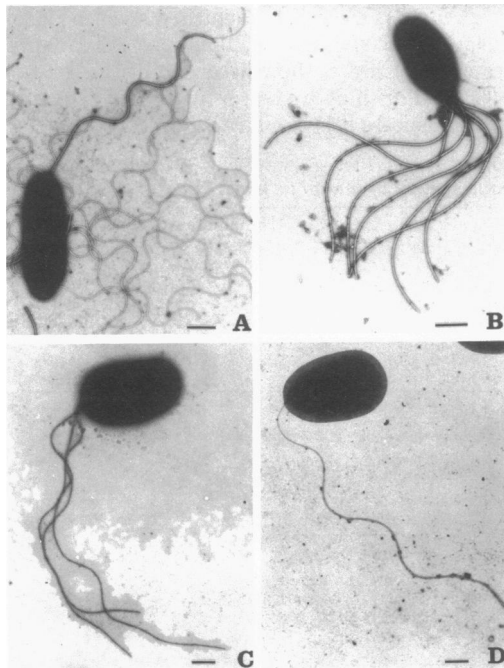


FIG. 3. Electron micrographs of negatively stained preparations of luminous bacteria. Bars = 0.5  $\mu$ m (A) *B. harveyi*, showing a single sheathed, polar flagellum and unsheathed peritrichous flagella. (B) *P. fischeri*, showing a tuft of sheathed flagella. (C) *P. phosphoreum*, showing a tuft of unsheathed flagella. (D) *P. leiognathi*, showing a single unsheathed polar flagellum. (These pictures were supplied by P. Baumann. Used with permission; from reference 78.)

*P. phosphoreum* from seawater samples exhibited some growth requirement, most commonly methionine. Of 500 *P. phosphoreum* strains isolated from the Atlantic Ocean at depths between 200 and 1,000 m Ruby et al. (E. G. Ruby, E. P. Greenberg, and J. W. Hastings, Appl. Environ. Microbiol., in press) found that 117 would not grow on minimal medium. Of these, 52 were chosen at random and tested; 51 grew on minimal medium supplemented with methionine. However, Ruby and Neelson (88) found no strains that required growth factors among approximately 2,300 fresh isolates from coastal waters at San Diego, Calif. Only 16 of these were *P. phosphoreum*; 651 were *B. harveyi*, and 1,601 were *P. fischeri*.

As mentioned above, a useful distinction between the two genera relates to the kinetics of the reaction of the luciferase when assayed in vitro. Although the luciferases from all species require the same components, the decay kinetics (enzyme turnover time) for the reaction in vitro using dodecanal are slow for luciferases from *Beneckea* and fast for those from *Photobacterium* (Fig. 3). Thus, the luciferase assay can be used for rapid screening at the generic level. Since this enzymatic pathway is apparently unique to bacteria, the existence of bacterial luciferase can be used as evidence for the presence of luminescent bacteria, even when they cannot be cultured (D. Cohn, G. Leisman, and K. H. Neelson, Abstr., Annu. Meet. Am. Soc. Photobiol. 1979). The reaction has also provided evidence for the presence of a functional luciferase gene even when luminescence in vivo is not detectable (Neelson and Walton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978).

#### Terrestrial and Freshwater Forms

The existence of nonmarine luminous bacteria, in particular the occurrence of a freshwater strain of *Vibrio albensis* (now called *Vibrio cholerae*, sometimes called *V. cholerae* biotype *albensis*), is indicated from the earlier literature (32). This strain has a low (50 mM) requirement for sodium ion and a DNA guanine plus cytosine content of 47.8 mol%; DNA-DNA hybridization studies indicate that it hybridizes 100% with the type strain of *V. cholerae*, whereas *Beneckea* strains hybridize poorly with *V. cholerae* (80). However, luciferase from this strain exhibits *Beneckea*-type (slow) kinetics (Kopecky and Neelson, unpublished data). Neither the distribution nor the physiology of luminous members of this species has been studied.

Recently, luminescence has been reported to occur in some members of a group of bacteria which are symbiotic with soil nematodes and collaborate with the nematodes in the parasiti-

zation of insects (52, 76; see below). The biochemistry of light emission is similar to that of the marine forms, and the reaction exhibits slow (*Beneckea*-like) kinetics (75). A new genus (*Xenorhabdus*) with luminous (*X. luminescens*) and nonluminous (*X. nematophilus*) members has been created to accommodate these bacteria (Table 1) (92). Members of this genus have a DNA guanine plus cytosine content of 43 to 44 mol% and share many properties with other members of the *Enterobacteriaceae*, in which group the genus is placed. The cells are unusually large for luminous bacteria (length, 5 to 10  $\mu\text{m}$ ), pigmented, and chitinase and oxidase negative, and they prefer low salt concentrations; growth is inhibited at 3% NaCl. Both luminous and nonluminous species produce antibiotic-like substances that presumably are responsible for the fact that infected dead insects do not putrefy (52). Several of these inhibitory compounds have been isolated and purified from the growth medium (B. Fenical, personal communication).

#### CONTROL OF THE SYNTHESIS AND ACTIVITY OF THE LUMINESCENT SYSTEM

Knowledge of control mechanisms is important to the understanding of the selective advantage of luminescence and the ecology of the bacteria. Most of the effects elucidated involve control of synthesis, and up to now there is no case known in which luciferase synthesis is affected without a concomitant effect on the synthesis of aldehyde (e.g., the biosynthesis of luciferase and the enzymes involved in aldehyde production are controlled in parallel). Thus, if luciferase synthesis is low, its ability to act in vivo is also lowered by virtue of substrate (aldehyde) limitation.

#### Autoinduction

The most obvious and perhaps the most significant physiological control of light emission involves control of the synthesis of the luminescent system by the bacteria themselves. This has been referred to as autoinduction. It was first described by Neelson et al. (69) and was postulated to involve a substance (autoinducer) that accumulates in the growth medium and induces the synthesis of the components of the luminescence system. This hypothesis is supported by the demonstration that both *P. fischeri* and *B. harveyi* cells produce a substance which induces bioluminescence at low cell density and which may be isolated and concentrated from the culture medium (20, 25, 63). Although the autoinducer is apparently a different compound in *Photobacterium* and *Beneckea* species

(the substances do not cross-react), many non-luminous *Beneckeia* species produce autoinducer activity in large amounts (some more than *B. harveyi*) which induces the luminescent system in *B. harveyi* (25). Several strains of *P. fischeri* have been found to differ in the amount of autoinducer produced (63); the amount of luminescence in vivo is correlated with autoinducer levels.

In a previous review (40) there was a report based on unpublished data that autoinducer from *P. fischeri* had a molecular weight of about 159 and properties similar to those of indole acetaldehyde. This is now believed to have been in error; the autoinducer was a minor contaminant in the fraction under study (A. Eberhard, personal communication). Separation of the active fraction has now been achieved, and its structure determination is under way (Eberhard, personal communication).

Support for the autoinduction hypothesis has been obtained recently from two types of studies. In the first, *P. fischeri* cells were grown in a chemostat in which the cell density was regulated by carbon (glycerol) limitation (R. A. Rosen and K. H. Nealson, Abstr. Annu. Meet. Am. Soc. Photobiol. 1979, p. 150-151). At steady-state cell densities of  $10^8$  ml<sup>-1</sup> or greater, the cells remained brightly luminous at an intensity

per cell independent of cell density (Fig. 4A). Below this density the luminescence per cell dropped sharply (by a factor of 100 with a 10-fold decrease in cell number). At cell densities below  $5 \times 10^6$  cells per ml no light emission was detectable. When purified autoinducer was then added, the luminescence increased by several orders of magnitude within hours (Rosson and Nealson, Abstr. Annu. Meet. Am. Soc. Photobiol. 1979). A different approach with *B. harveyi* provided a similar conclusion (J. W. Hastings and S. Ulitzur, Abstr. Annu. Meet. Am. Soc. Photobiol. 1979, p. 66). By repeated dilution, cells were maintained at densities below  $10^7$  ml<sup>-1</sup>; luminescence per cell rapidly decreased to less than 1% of that seen after induction (Fig. 4B).

Autoinduction thus occurs in members of two different marine genera and may occur in all, since similar density-dependent effects upon the development of luminescence have been observed in all luminous species. However, it has been reported that strains of both *P. phosphoreum* (96) and *P. leiognathi* (48) exhibit constitutive luciferase synthesis, and autoinducer has not actually been isolated from either of these species or from *Xenorhabdus* species.

It thus appears that at low cell densities or under ecological conditions where the autoinducer cannot accumulate, luminous bacteria are

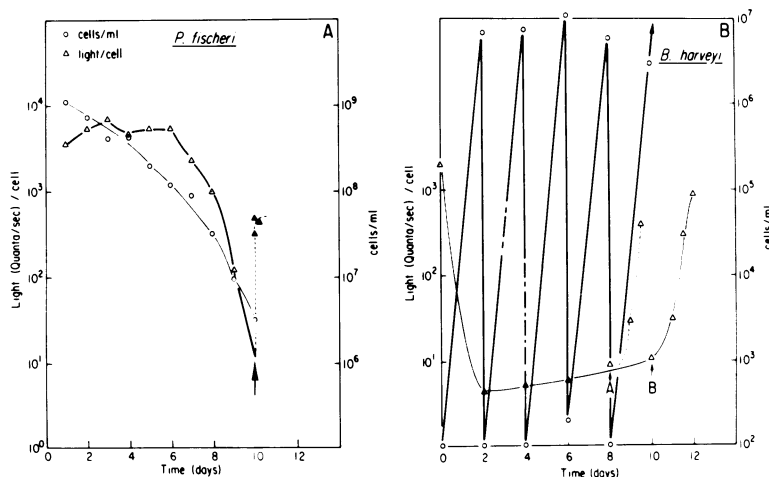


FIG. 4. Loss of luminescence in cells growing at low cell densities. (A) Chemostat experiment with *P. fischeri*. Each day the carbon (glycerol) concentration was lowered by a factor of two, and the cells of *P. fischeri* were allowed to grow at that concentration. When the cell density dropped below about  $10^8$  cells per ml, the luminescence per cell decreased; at a cell density of  $3 \times 10^6$  cells per ml, light emission was undetectable, i.e., less than 10 quanta/s per cell. Addition of purified autoinducer (arrow) restored bioluminescence in the culture at this density. (B) Cells of *B. harveyi* were maintained at low density by repetitive subculturing rather than in a chemostat; no nutrient limitation was used. When the culture reached a density of about  $10^7$  cells per ml, it was diluted to approximately  $10^2$  cells per ml in the same medium. After the first dilution, luminescence per cell fell to and remained at a low level through all subsequent dilutions. Either the addition of inducer (arrow A) or allowing the cells to continue growth (arrow B) resulted in the induction of bioluminescence.

repressed for luciferase synthesis and are thus nonluminescent. This consideration is especially applicable to the case of bacteria growing unconfined, as, for example, free in seawater. Kelly and Tett (49) have proposed that luminous bacteria might provide a background glow in the ocean. Direct observations are lacking, and this would not be expected to occur unless the bacterial counts are very high, the water contains autoinducer, or a bacterial strain that does not require autoinducer is involved. One test of the hypothesis was reported by Booth and Nealson (C. K. Booth and K. H. Nealson, *Biophys. J.* **15**: 53a, 1975). Light intensity measurements of seawater samples known to contain luminous bacteria (by subsequent plating) showed that the bacteria were nonluminescent or that the luminosity was at least 100-fold less than the light intensity of the same cells after growth in the laboratory.

On the other hand, autoinduction presumably occurs for associated bacteria, whether they are light organ symbionts, parasites, gut bacteria, or saprophytes. In such cases, cell density becomes high so that autoinducer can accumulate to levels sufficient for the induction of the luminescence system. It should be stressed here that since the control by autoinduction is at the level of transcription (63, 69), response to autoinducer can be measured only after some minutes. Thus, it is not a mechanism that a host fish might exploit for the immediate control of luminescence from the light organ. On the other hand, for long-term situations, such as conserving energy during day-night cycles, it is not inconceivable that the level of bacterial luminescence could be modulated by a host fish interacting at the level of autoinduction. Such ideas have never been tested.

### Catabolite Repression

Inducible enzymes are synthesized and have a function under some conditions but not under others (56). For many such enzymes induction is mediated by some specific nutrient for which catabolic enzymes are not produced constitutively. Moreover, the induced synthesis of the relevant enzyme is often repressed by glucose, even in the presence of inducer, and this repression can be overcome by exogenous cyclic adenosine monophosphate (cAMP); glucose repression and reversal by cAMP are referred to as catabolite repression. The synthesis of the luminescence system in *B. harveyi* is subject to catabolite repression (66). Furthermore, cAMP binding protein occurs in large amounts in *B. harveyi*, and it is immunologically homologous with the cAMP binding proteins of *Escherichia coli* and several other enteric bacteria (73). By

analogy, luciferase in *B. harveyi* has been hypothesized to be a nonessential enzyme, which is functional under certain, presumably advantageous, conditions and repressed under others.

*B. harveyi* mutants that are resistant to catabolite repression have been isolated as "bright on glucose" phenotypes, but are not yet well characterized (P. Lin, M. H. Saier, Jr., and K. H. Nealson, *Fed. Proc.* **35**:1361, 1976; K. H. Nealson and J. W. Hastings, *Bacteriol. Proc.*, p. 219, 1970). Ulitzur and Yashphe (94) isolated a mutant that is nonluminescent unless supplied with exogenous cAMP. As expected, the mutation is pleiotropic; the strain is deficient for a variety of other functions for which cAMP is normally required in enteric bacteria (56).

The *Photobacterium* species are quite different, but not yet fully characterized. Henry and Michelson (42) reported that a strain of *P. leiognathi* displays a strong permanent glucose repression that is not reversed by cAMP. Makemson (57) reported glucose repression that is not reversed by cAMP in *P. phosphoreum*. *P. fischeri* exhibits a transient repression of the development of luminescence when switched from glycerol- to glucose-containing media. This effect is not reversible by cAMP, and once the cells are glucose adapted, no further effect is seen (86). *Xenorhabdus* species have not been studied in this regard.

### Composition of the Growth Medium

In addition to control by catabolite repression, the synthesis of the luminescence system can be dramatically affected by the composition of the growth medium. In *B. harveyi*, growth in a minimal medium with glycerol, which does not repress the luminous system, is much slower than growth in a complete medium, and the bioluminescence is severely repressed. Although a lag in the development of luminescence that is characteristic of autoinduction occurs, only very small amounts (1% or less) of luciferase are actually produced (69). Under these conditions, additional autoinducer does not increase luciferase synthesis or luminescence, but arginine and the salt (NaCl) concentration do have pronounced effects.

Added arginine dramatically stimulates the development of luminescence of *B. harveyi* growing in a minimal medium (17, 69, 98). Citrulline and argininosuccinate (the immediate biosynthetic precursors of arginine) also stimulate luminescence, as does proline, but to a lesser degree; the mode of action is not known. In addition, *B. harveyi* is considerably brighter when grown in minimal medium with low salt (1 instead of 3% NaCl) (K. H. Nealson, Ph.D. thesis, University of Chicago, Chicago, Ill., 1969;



C. A. Waters, Ph.D. thesis, Harvard University, Cambridge, Mass., 1974). In some nonluminous *Beneckea* species that contain luciferase, its synthesis is also enhanced by growth in a medium with low salt (Nealson and Walton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I131, p. 102). Although the salt effect has not been investigated in detail, it may be related to the arginine effect, since cells grown in low salt exhibit less stimulation by arginine. Furthermore, mutants that have escaped the arginine requirement and are thus bright when grown on a minimal medium without arginine exhibit little or no enhancement of luminescence when grown in low salt. Neither the salt effect nor the arginine effect occurs in a complete medium (Nealson, Ph.D. thesis; Waters, Ph.D. thesis).

Whether these phenomena occur in the other luminous species and, if so, to what extent is not known. From recent studies it appears that light emission per cell is also quite low for *P. leiognathi* grown in a minimal medium (Rossen and Nealson, Abstr. Annu. Meet. Am. Soc. Photobiol. 1979) and that this is a species that grows well under conditions of low nutrients (100).

### Oxygen

When stab cultures of different species of luminous bacteria are examined, two different patterns of growth and luminescence are seen. In some (*B. harveyi* and *P. leiognathi*), luminescence is maximal at the surface of the agar, where oxygen concentration is high. In others (*P. fischeri* and *P. phosphoreum*), maximal luminescence occurs deep in the stab, where growth is inhibited and oxygen concentration is low (67). Analysis of this phenomenon has shown that oxygen is important as a controlling element in the synthesis of the luminescence system. For *P. fischeri* and *P. phosphoreum*, at low concentrations where growth is limited, luciferase synthesis is not halted, resulting in cells with high luciferase content. Quite a different response occurs in *B. harveyi* and *P. leiognathi*, in which there is a tight coupling between growth and luciferase synthesis. Both properties are simultaneously limited, so that cells grown under oxygen-limiting conditions develop the same amount of luciferase as do well-aerated cells at the same cell density.

These differences may have clear ecological implications and suggest that the different species occupy niches that are different with regard to oxygen concentration or the necessity for the presence of the luminescence system or both. For instance, the light organ may represent a niche where the oxygen concentration under which the bacteria are maintained could be controlled by the host fish. Experiments in this area

may lead to significant advances in the understanding of the ecology of these bacteria.

### Pyruvate and Excretion Products

When *P. fischeri* is grown in a medium containing glucose or one of several other sugars as the carbon source, pyruvate accumulates in the growth medium (87; Ruby, Ph.D. thesis). Unless the medium is strongly buffered, the pH drops and luminescence ceases. *P. fischeri* grows with pyruvate as the sole source of carbon, so its excretion during growth on other substrates is unusual. It may simply be a case of unbalanced growth, but the mechanism has yet to be elucidated. Pyruvate excretion has also been observed in *Beneckea* species (both luminous and nonluminous) and *P. phosphoreum*, but not in *P. leiognathi*. Whether pyruvate excretion is ecologically important to the luminous bacteria under any conditions or is an artifact of the laboratory studies awaits the demonstration of pyruvate excretion under *in situ* conditions. However, nutrient exchange is a common feature of many symbioses, and pyruvate may represent such a nutrient, as proposed for one model of symbiosis of luminous bacteria with luminous fish (65, 86).

### Dark (K) Variants of Luminous Bacteria

No review of the physiology of luminous bacteria is complete without consideration of a confusing and poorly understood property noted and studied by Beijerinck at the turn of the century (10, 11); namely, luminous bacteria have the capacity, indeed the proclivity, to form spontaneous dark variants (51). Many years later, in a class experiment regularly included in the summer course of Van Niel at Pacific Grove, such dark strains of luminous bacteria were isolated. Bright, freshly isolated luminous bacteria, when subcultured at high temperatures, reliably yielded dark variants. The interest of Van Niel in this related, at least in part, to his experience as the curator of the Delft culture collection. There he had repeatedly encountered the appearance of dark strains in his cultures of luminous bacteria and was obliged to reisolate single bright colonies upon each transfer (C. B. Van Niel, personal communication).

We have conducted some studies with spontaneous dark variants of *B. harveyi*, which are referred to as K variants. If a liquid culture is maintained without shaking for several days after it reaches stationary phase, it is common to find K variants (51). Similar dark variants are also readily isolated after treatment with acridine dyes (68; Nealson, Ph.D. thesis). The K variants are not completely nonluminous; all

exhibit a dim light emission, the exact level of which may vary between  $10^{-2}$  and  $10^{-5}$  of the wild-type level. Thus, they do not lack the luciferase gene; they simply express it at extremely low levels. They are low in luciferase, in antigenically active luciferase cross-reacting material (CRM), and in all or many of the other components of the luminescence system (60). Failure to synthesize the luminescence system is not attributable to the lack of autoinducer production, nor is luciferase synthesis stimulated by arginine or cAMP.

The K variants are genetically stable and have several other altered properties. In addition to their deficiencies in bioluminescence, they are altered in colonial morphology, in flagellation, and in phage sensitivity. The wild-type colonies are convex, creamy, and translucent, whereas the K-variant colonies are flat and more transparent. The K variants are also sensitive to anaerobic conditions; they die rapidly, whereas the wild-type cells survive well (Nealson, Ph.D. thesis). Such conditions are the only ones that have been found that result in selection of bright bacteria over darks.

Beijerinck (10, 11) noted that dark variants occasionally reverted back to the wild-type bright state. When this occurs, all of the altered characteristics revert simultaneously (51; Nealson, Ph.D. thesis). The appearance of dark forms is more frequent in freshly isolated strains, even at room temperature, compared with laboratory strains that have been subcultured repeatedly (62). In cultures of either bright or dark forms maintained under conditions of rapid growth or vigorous aeration or both, the appearance of the alternate form is not common, whereas partial anaerobiosis (as in dense cultures without aeration) and higher temperatures appear to favor the appearance of variant forms. Neither genetic transfer between bright forms and dark forms nor a specific inhibitory or toxic factor that might be produced by either form could be demonstrated (Nealson, Ph.D. thesis). These phenomena have not been studied in detail, and the mechanism by which dark variants are formed and by which reversion occurs is not understood.

In other species of luminous bacteria, the formation of K variants is known, but has not been studied. Many dim and dark variants have been isolated from *P. leiognathi* (39) and *P. phosphoreum* (B. M. Tebo and K. H. Nealson, unpublished data), but their properties are not well defined. Understanding the tendency of the various species to form dark variants and the factors that control the reversible transformation between bright and dark forms is necessary if the ecology of the luminous bacteria is to be understood.

## HABITATS AND DISTRIBUTION OF LUMINOUS BACTERIA

Luminous bacteria are widely distributed in many different marine habitats, whereas they are less abundant in nonmarine environments (32). A tabulation of these habitats and associations as they are now known is presented in Table 2.

### Free Living

It is well documented that luminous bacteria are ubiquitous in seawater as free-living forms, but only recently has it been shown that most are unattached and that the free-living populations are dynamic and change in a regular fashion (88) (Fig. 5). Coastal waters near San Diego, Calif., regularly contained between  $1 \times 10^3$  and  $6 \times 10^3$  luminous bacteria per liter over a 2-year sampling period. In the summer, the luminous isolates were predominantly *Beneckeia* spp., whereas in the winter *P. fischeri* was more abundant. Summer and winter populations at Woods Hole, Mass., showed similar seasonal patterns (Ruby and Hastings, unpublished data).

Like many marine heterotrophic bacteria (102), the luminous forms occur at a much lower abundance in the surface waters of the open ocean than in coastal waters. In a recent quantitative study (Ruby et al., Appl. Environ. Microbiol., in press) it was found that in open ocean surface waters at several stations in the eastern Atlantic Ocean, *B. harveyi* showed marked seasonal changes, ranging from 2 to 300 colony-forming units per liter. In the same study, the species distribution as a function of depth was also examined. Midwater samples (100 to 1,000 m) in certain areas of the Atlantic Ocean contain *P. phosphoreum* in relatively high numbers (20 to 100 cells per liter) irrespective of season and very few of any of the other luminous species. Since *P. phosphoreum* does not occur (less than 1 cell per liter) in the surface waters in those areas, there is an actual increase in the numbers of this species with depth, as both temperature and (presumably) dissolved nutrient levels decrease (Fig. 6). The numbers of luminous bacteria decrease below 1,000 m. The reason for the abundance of *P. phosphoreum* in the midwater region is not known, but it may be related to the association of luminous bacteria with higher organisms, especially fish, either as light organ or gut symbionts or both. Such associations would result in the constant release of the bacteria into the seawater.

Other data are limited. Samples of seawater from the Arctic Ocean (J. Baross, personal communication) and Antarctic Ocean (72) are reported to contain primarily *P. phosphoreum* and

TABLE 2. Habitats and associations of luminous bacteria

Mode	Habitat or host	Species
<b>Nonsymbionts</b>		
Free living	Seawater	All <i>Photobacterium</i> and <i>Beneckea</i> spp.
	Soil (?), freshwater (?)	<i>X. luminescens</i> (?)
Saprophytic	Marine animals, fish, salt meat(?)	All <i>Photobacterium</i> and <i>Beneckea</i> spp.
	Wounds, meat	<i>X. luminescens</i> (?)
<b>Nonspecific symbionts</b>		
Commensal	Digestive tracts, marine fish and invertebrates, outer surfaces of marine animals	All <i>Photobacterium</i> and <i>Beneckea</i> spp. <sup>a</sup>
Parasitic	Marine crustacea	<i>P. fischeri</i> , <i>P. phosphoreum</i> , <i>B. harveyi</i>
	Terrestrial and freshwater animals	<i>X. luminescens</i> , others cultured, but not identified
<b>Specific symbionts</b>		
Symbiotic/parasitic	Nematode/caterpillar	<i>X. luminescens</i>
Light organ exosymbionts	Teleost fishes (11 families)	<i>P. fischeri</i> , <i>P. phosphoreum</i> , <i>P. leiognathi</i> ; some isolates not identified, some not cultured
	Squid	
	( <i>Euprymna scolopes</i> )	<i>P. fischeri</i> <sup>b</sup>
	( <i>Heteroteuthis hawaiiensis</i> )	Not cultured
Light organ endosymbionts <sup>c</sup>	Tunicates, squid(?)	Not cultured or identified

<sup>a</sup> *B. splendida* and *P. leiognathi* have not been rigorously demonstrated in such habitats, but their presence seems certain.

<sup>b</sup> The older literature (12, 32) contains reports that isolates cultured from squids exhibit characteristics that are very different from "ordinary" marine luminous bacteria. Several different names were assigned, including *Vibrio pierantonii*, which was later identified as *P. fischeri* (78). This has been confirmed with recent isolates from *Euprymna scolopes* (G. Leisman, unpublished data).

<sup>c</sup> The occurrence of luminous endosymbionts has not been firmly established.

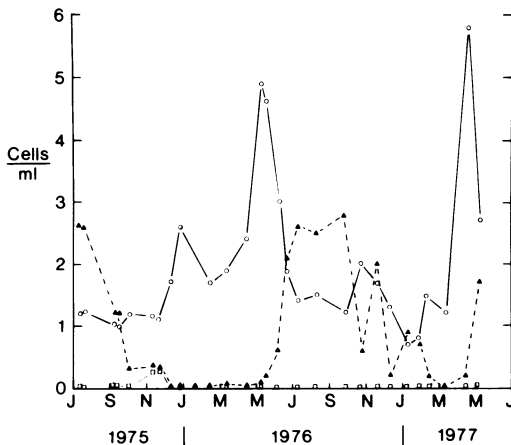


FIG. 5. Abundance of species of luminous bacteria in surface seawater collected at the entrance of Mission Bay (San Diego, Calif.) on incoming tides at 3- to 4-week intervals. Symbols:  $\blacktriangle$ , *B. harveyi*;  $\triangle$ , *P. fischeri*;  $\square$ , *P. phosphoreum*. Each sample was based on taxonomic identification of between 80 and 100 isolates (64).

*P. logei*, but how these are distributed vertically and seasonally is not yet documented. Yetinson and Shilo (100) have reported the patterns of variation for the luminous bacteria in coastal surface waters of the Mediterranean Sea near

Tel Aviv, Israel, and for the Gulf of Elat. Some of the patterns are complex, involving *B. harveyi*, *P. leiognathi*, and *P. fischeri*, although in a saline lagoon only *B. harveyi* was present. These authors postulate that several bacterial properties dictate the distribution of the species, including sensitivity to photoinactivation and thermal inactivation, resistance to hypersalinity, and the ability to grow under nutrient-poor conditions (89).

The occurrence of luminous bacteria in the nematode-caterpillar relationships suggests that these species might also occur as free-living soil bacteria (Table 2), but they have never been reported as such.

#### Saprophytic Forms: Natural Enrichments

If a swab from the surface of a marine animal, especially a fish or a crustacean (but not a plant or inanimate surface), is streaked onto seawater nutrient agar plates, luminous colonies almost always arise in numbers exceeding those that might be obtained by plating seawater directly. The time-honored method for isolating luminous bacteria involves allowing bacterial growth on the surface of freshly killed fish or squid, followed by inspection and visual selection in the dark for luminous colonies. Both *Beneckea*

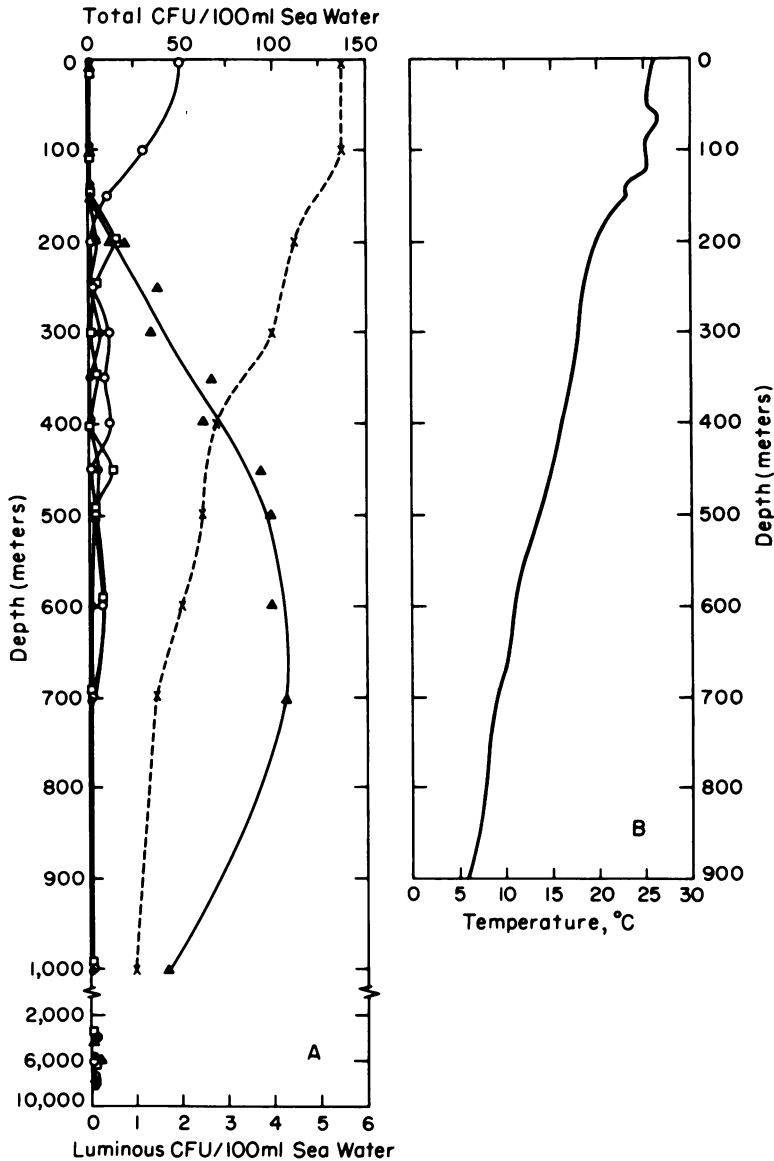


FIG. 6. Bacterial colony counts and water temperatures in the water column over the Puerto Rico Trench. (A) Abundance of planktonic bacteria as a function of depth. Symbols: x, total colony-forming units (CFU);  $\blacktriangle$ , *P. phosphoreum*;  $\square$ , *P. leiognathi*;  $\bullet$ , *P. fischeri*;  $\circ$ , *Beneckeia* spp. Data are from three hydrocasts made over a period of 4 days at two stations. (B) Temperature profile of the water at the time of the hydrocasts.

and *Photobacterium* species may be obtained in this manner; the temperature of incubation may result in enrichment for one or another of the different species (see above).

Meat, in general, is susceptible to overgrowth by luminous bacteria. Salted hams and other such stored meats have commonly been observed to emit light due to a growth of luminous bacteria (32). Such observations have been less frequent with the advent of refrigerators, espe-

cially those with automatic lights. The bacteria are reputed to be harmless, in fact to be an indication that no putrefaction has occurred. There are also older accounts of the occurrence of luminous bacteria in open human wounds, reported especially from battlefield hospitals during the 19th century (33); the presence of luminous bacteria was taken as a good sign of wound healing. It has always been tacitly (sometimes explicitly) assumed that the bacteria in-

volved in these associations were typical marine forms. In fact, no isolates have been studied, so there is no knowledge of their characteristics. With the discovery of the terrestrial genus *Xenorhabdus*, it now seems possible that some of the above cases may involve nonmarine luminous bacteria. The fact that *Xenorhabdus* isolates produce antibiotics is especially interesting in connection with the report that there was no putrefaction in the presence of the luminous bacteria in question.

### Commensal Forms

Quantitatively, the most important habitat of luminous bacteria may be that of the gut tracts of marine animals. It is not uncommon to find between  $5 \times 10^6$  and  $5 \times 10^7$  luminous colony-forming units per m in the gut material of certain marine fishes (85), and it may be that many such fishes carry these bacteria as major enteric populations. All species of marine luminous bacteria produce extracellular chitinase (78, 90) and are thus conceivably important as gut symbionts in the digestion of chitin (103; B. H. Robison and J. G. Morin, Abstr. Annu. Meet. West. Soc. Natural. 1977, 71a). Baross (unpublished data) found that the gut contents of flatfish (*Parophrys vetulus* and *Citarichthys sordidus*) contained about  $10^8$  bacteria per g, all of which were luminous and chitinoclastic. Ruby and Morin (85) studied *Oxyulis californica*, *Chromis punctipinnis*, and *Argyropelecus hemigymnus* from the waters off southern California and found *B. harveyi*, *P. fischeri*, and *P. phosphoreum* in different samples, with often just one of the species predominating; the occurrence in the fish was often correlated with the species composition of the planktonic luminous bacterial population. Fecal pellets were luminescent and also contained viable luminous bacteria and extractable luciferase (85). Luminous fecal pellets have also been reported from Antarctic cod (77) and from a midwater shrimp (95) although no isolation of luminous bacteria was reported in either case. Hastings and Marechal (unpublished data) plated and counted bacteria from intestinal tracts of fish (*A. hemigymnus* and *Myctophum*) coming from deep water in the Straits of Messina, Sicily. In different samples 40 to 100% of the colony-forming units were luminous and were a single species, *P. phosphoreum*. Invertebrates, such as squid, mussels, scallops, and crabs, also carry such bacteria (32; J. Baross, P. A. Tester, and R. Y. Morita, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, 11, p. 230; P. Dunlap, unpublished data).

### Parasites

**Marine.** Although there are few recent studies on the subject, it is well known from the older literature that luminous bacteria can infect the tissues of a variety of marine crustaceans (24, 32, 46). It has usually been possible to isolate and grow the infecting organism in pure culture, and the infections are apparently not species specific. Different species of luminous bacteria occur as parasites, and the same host may be parasitized by different bacterial species (both *Photobacterium* and *Beneckeia*); the parasitism is apparently correlated with season, corresponding to the abundance of free-living isolates (Nealson, unpublished data). Moreover, parasitic bacteria apparently have a wide host range and are capable of infecting many different crustaceans (32). Luminous bacteria grow in the hemolymph of the infected animals, often making them easily visible. Whether an intermediate host, such as is found in the nematode-caterpillar case (see below), is involved is not known.

There are current studies concerned with necrotic lesions in the Tanner crab exoskeleton infected specifically with *P. phosphoreum*. The extracellular bacterial chitinase may provide a mechanism for the bacteria to inhabit this niche (J. Baross, personal communication).

**Nonmarine.** There are many reports in the older literature of parasitic infections of terrestrial organisms by luminous bacteria; the well-documented hosts include mole crickets, mayflies, ants, wood lice, and millipedes (32). As mentioned above, recent studies link the parasitism of caterpillars by luminous bacteria (*X. luminescens*) with a mutualistic symbiosis between the bacteria and a specific genus of nematode (52, 76). The luminous bacteria are carried symbiotically in the gut of a nematode and occur there in small numbers, estimated to be less than 100 cells per animal (G. Thomas, personal communication); the nematode stage is not visibly luminous. In its juvenile stage the nematode is ingested by the caterpillar and bores through the gut wall, inoculating the hemolymph with the bacteria, which then grow there and are pathogenic for the caterpillar. As mentioned above, the bacteria produce an antibiotic that presumably prevents putrefaction. The life cycle of the nematode is completed in the hemocoel, and luminous bacteria are incorporated into the progeny. Poinar (personal communication) has proposed that the function of the luminescence is to aid in the success of the nematode progeny by attraction of additional potential hosts (other caterpillars). It seems equally likely that the light could aid in the dispersion of the nematode

progeny via animals that are attracted by the luminescence and feed on the caterpillars. Free luminous bacteria ingested by the caterpillars are not capable of killing the hosts, and without the bacteria the nematode cannot complete its life cycle (52, 76). In fact, bacteria-free nematodes are not pathogenic. However, the bacteria may be cultured and are highly virulent when injected directly into the hemocoel; as few as one or two bacteria so injected are sufficient to kill a caterpillar. The bacteria, classed in the newly created genus *Xenorhabdus* (92), have properties differing strikingly from those of *Photobacterium* and *Beneckea* species (52, 76), but the luciferase and light-emitting reactions are similar (75).

It is not possible to state whether the other examples of terrestrial and freshwater animals being infected by luminous bacteria (25, 32) are due to similar nonmarine bacteria, but without inquiry, they should certainly not be assumed to be marine forms.

**Light Organ Symbionts**

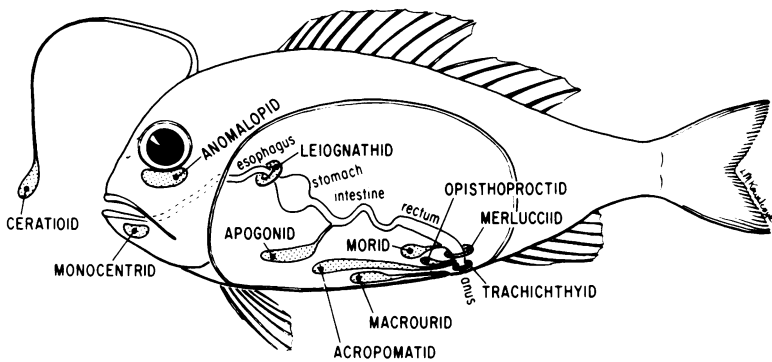
**Teleost fishes.** Members of at least 30 genera in 11 families of teleost fishes possess specialized light organs in which luminous bacteria occur (Fig. 7). It should be recalled that there are many other luminous fishes (in approximately 25 families) that generate their own light and do not utilize luminous bacteria (32, 45).

The bacteria that have been cultured from

fish light organs are all members of the genus *Photobacterium* and are species specific for a particular host. The tropical and temperate fishes, which occur in shallow and warmer waters, harbor *P. leiognathi* and *P. fischeri*, which tolerate higher temperatures. The deep-sea and midwater fishes, on the other hand, harbor psychrotrophic *P. phosphoreum* species as their symbionts (73). *P. logei* is a newly identified psychrotrophic species that has not yet been reported to be specifically associated with a higher organism (3).

Not all of the symbionts have been identified, however; in some cases (Table 2) luminous bacteria that have been visualized in light organs have proven impossible to culture (see below). Species determination of these forms has not been possible, but detection of bacterial luciferase has indicated that bacteria are indeed present. At least one group, the anomalopid fishes, have luciferase with slow decay kinetics, suggesting that their symbiotic bacteria could be of the *Beneckea* type (Cohn et al. Abstr. Annu. Meet. Am. Soc. Photobiol., 1979).

In the symbiotic association mutual benefits accrue; the bacteria are supplied with nutrients and a protected environment, whereas the fish is supplied with light. The function of the light may be classed under one or more of three main categories: attracting prey, assisting in escaping or diverting predation, and communication (61). For example, angler fish (Lophiiformes: Cera-



<i>P. phosphoreum</i>	<i>P. fischeri</i>	<i>P. leiognathi</i>	Non-culturable	Not identified
Macrourid	Monocentrid	Leiognathid	Ceratioid	Acropomatid
Merlucciid		Apogonid	Anomalopid	
Morid				
Opisthoproctid				
Trachichthyid				

FIG. 7. The "ichthylicht". A diagrammatic fish is used to indicate the approximate locations, sizes, and openings of the light organs of the several different families of luminous fishes that culture symbiotic luminous bacteria as a source of light for the organ. The taxa of bacteria known to be specifically associated with each fish group are listed at the bottom of the figure.

tioida) possess an illuminated fishing lure-like structure that may attract would-be predators, which are then converted into prey (71, 74).

Light emission is used to avoid predation in a variety of ways, including frightening and diverting predators. Another technique to avoid predation, uniquely possible with light emission, involves counterillumination, namely ventral emission of light during the day, the intensity and color of which matches the down-welling light that would otherwise silhouette the fish (14, 36, 58, 95). The preponderant ventral location of luminescence in many families suggests that this may be its function in these cases, and the hypothesis has been supported experimentally (13, 95, 101). In the case of the pony fish (Leionathidae) the light organ is located deep within the body as an outpocketing of the esophagus, and highly adapted optical mechanisms allow this light to be presented as a uniform ventral glow (36). Some of the light is initially directed into the swim bladder, which is internally reflective, from whence it passes to the entire posterior ventral surface by means of reflective and light-conducting fibers and tissues. There are many variations on this theme in the Leionathidae.

Fish may also use the luminescence from light organs for intraspecies communication and to illuminate their surroundings, as in the flashlight (anomalopid) and pinecone (monocentrid) fishes.

Within the organs, the bacteria emit light continuously, as they do in laboratory culture, and in all cases the control of light emission involves some specific host mechanism, which may be a shutter, the rotation of the organ, or chromatophores (45). Figure 8 shows progressively higher magnifications of the light organ of *Monocentris japonica*, illustrating the several different components of the organ. The bacterial symbionts are extracellular and are maintained in canals or tubules which communicate with the exterior either directly (monocentrid, anomalopid, ceratioid, and macrourid fishes) or via the intestinal tract (45). The bacteria are characteristically densely packed within the tubules ( $10^9$  to  $10^{10}$  cells per ml of organ fluid); in the leionathids and monocentrids, microscopic and viable counts give similar values, indicating that nearly all of the cells are viable (39, 91). The uniform microscopic appearance of the bacteria in light organs and of the colonial isolates from these organs suggested that the bacteria are a single species (4, 5, 28); this has been confirmed by taxonomic analysis, which has shown, however, that different colonial isolates from a given organ may exhibit some different, but taxonomically inconsequential, specific characteristics

(21, 82, 84, 96).

Only a few of the numerous types of light organs have been described in detail. In all known cases, the organs are extensively vascularized, presumably for the supply of nutrients and oxygen to the bacteria; control of the level of the latter may be of physiological significance. An interesting feature revealed by recent ultrastructural studies of some light organs is the occurrence of large rather unusual mitochondrion-containing cells (Fig. 8). These have been observed in both anomalopids (50) and monocentrids (91), but they apparently do not occur in leionathids (5). Their function may relate to the maintenance of a low or proper oxygen tension (50), to the removal of bacterial excretion products, or to energy generation, as proposed by Nealson (65) in a model for symbiosis in the Monocentridae.

**Squid.** Most luminous squid have their own chemical mechanisms; only 2 (Loliginidae and Sepiolidae) of the 19 luminous families have genera that use luminous bacteria as the source of light (44). The luminous bacteria have been cultured from some but not all species. In the older literature various names were assigned that cannot be matched with current nomenclature (Table 3). The bacteria isolated from *Euprymna scolopes* have recently been identified as *P. fischeri* (G. Leisman, unpublished data).

In the well-described cases, the anatomical adaptations for the bacterial symbionts include paired organs in the mantle cavity, which lie against the ink sac near the anus (12, 53). Although the light is commonly emitted as a luminous cloud, in at least some species it can be seen in the animal itself through the body wall (43). The luminescence is presumably used to frighten and confuse other organisms; in the aphotic deep sea the usual black ink would of course be ineffective.

**Nonculturable symbionts.** There are many instances in which suspected bacterial symbionts have not been growable in host-free culture. For example, the symbionts of the anomalopid and ceratioid fishes have never been cultured, even though luminous bacteria have long been believed to be involved in light emission, based on both structural and physiological observations (29), but impossible to culture. Dilly and Herring (18), on the basis of morphological studies and the failure to culture luminous bacteria, concluded that the luminescence of the squid *Heteroteuthis dispar* was probably not bacterial in origin. For some such cases, it has now been possible to demonstrate conclusively, by biochemical analysis of light organ extracts, whether a bacterial system is involved (Cohn et al., Abstr. Annu. Meet. Am. Soc. Photobiol.

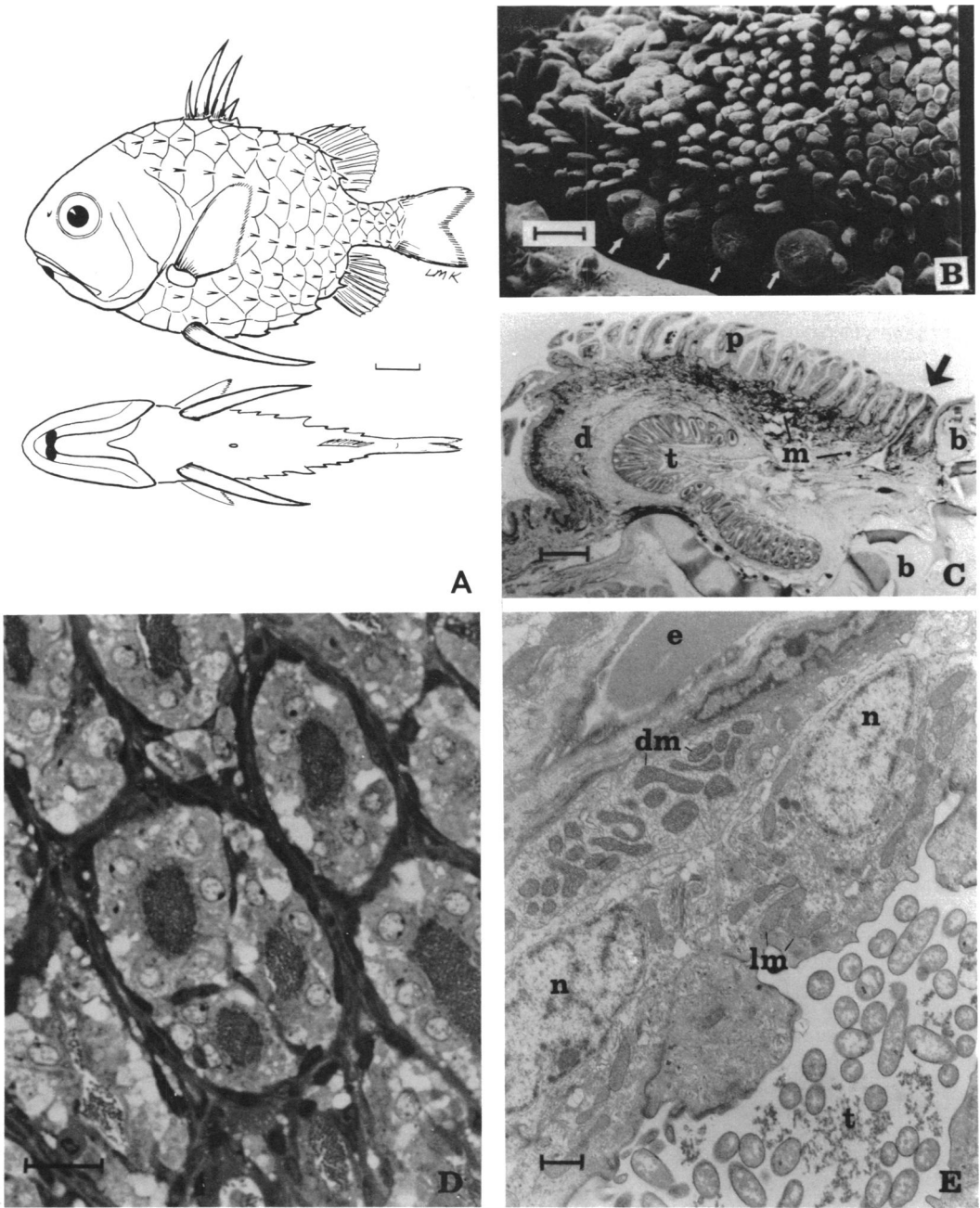


FIG. 8. Structural features of the luminous organ of the luminous fish *M. japonicus*. (A) Line drawing of fish and ventral view of lower jaw, showing the location of the light organs (solid black area). Bar = 1.0 cm. (B) Scanning electron micrograph of the dorsal surface of the light organ. Numerous dermal papillae can be seen. The emissary ducts from the light organ emerge at the tips of the four large papillae (arrows). Bar = 0.2 mm. (C) Light micrograph of a sagittal section of the lower jaw. m, Melanocytes; t, tubules with bacteria; b, mandibular bone; d, dermal layer; p, dermal papillae. The arrow points to an emissary duct. Bar = 50 μm. (D) Light micrograph showing the tubules of the light organ filled with bacteria. Tubules are lined with a single layer of cuboidal epithelial cells that display loose nuclear chromatin and prominent nucleoli supported by connective tissue cells. Blood capillaries are sparse and not readily visible. Bar = 15 μm. (E) Electron micrograph showing the major features of the tubule epithelium. Epithelial cells that make up the lining of the tubules have light-staining mitochondria (lm) with fine cristae. Epithelial cells that are further away from the tubule lumen next to the blood capillaries have dark-staining mitochondria (dm) with thick cristae. t, tubule with luminous bacteria; e, erythrocyte visible in capillary; n, nuclei of tubule epithelial cells. Bar = 1 μm.



TABLE 3. Bacterial luciferase assays in extracts of light organs of eucaryotes

Organism	Luciferase kinetics <sup>a</sup>
Cephalopod (squid) ( <i>Heteroteuthis hawaiiensis</i> )	Fast
Teleost (anomalopid) fishes	
<i>Photoblepharon palpebratus</i> <sup>b</sup>	Slow
<i>Anomalops katoptron</i> <sup>b</sup>	Slow
<i>Kryptophanaron alfredi</i>	Slow
Teleost (ceratioid) fishes	
<i>Cryptosavus couesii</i>	Fast
<i>Ceratius holboelli</i>	Fast
<i>Melanocetus johnsoni</i>	Fast
<i>Oneirodes</i> sp.	Fast

<sup>a</sup> Rate of decay of luminescence in the nonturnover assay. See Fig. 2.

<sup>b</sup> Previously reported to give light emission in the bacterial luciferase assay (29) but with no data on kinetics.

1979) (Table 3). It is of interest that the luciferases from the anomalopids exhibit slow kinetics, since all other marine symbionts possess luciferases with fast kinetics. Perhaps the anomalopids are symbiotic with a different bacterial group, more closely related to the *Beneckeia* species. This would be of particular interest ichthyologically, since the Anomalopidae is placed in the Beryciformes (83) along with the Monocentridae and Trachichthyidae, both of which culture *Photobacterium* species. Since bacterial luciferase is unique to procaryotes, its presence can also be used to implicate the involvement of symbiotic luminous bacteria in light emission (Cohn et al., Abstr. Annu. Meet. Am. Soc. Photobiol., 1979). The nonculturable symbiotic bacteria are evidently highly host dependent, which is possibly related to nutritional or other factors required for growth. This may represent an intermediate stage in the evolution of endosymbiosis.

**Endosymbionts.** Harvey (32) discusses but largely discounts the possibility that luminous tunicates harbor endosymbiotic bacteria as their source of light. Both pyrosomes and salps contain bacteria-like bodies; however, although many attempts have been made, bacteria have not been cultured from these organisms. Büchner (12) stated that symbiotic luminous bacteria are definitely involved and that they are large (2 to 3  $\mu\text{m}$  by 10 to 30  $\mu\text{m}$ ) sporeformers. The spores are reported to be intracellular, and it has been suggested that they are responsible for the infection of eggs and the transmission of the symbionts. Both their size and their capacity to form spores indicate that these bacteria would be in a different group altogether and that if such a symbiotic association occurs in tunicates, it would be fundamentally different from other

systems. Both Herring (43) and Galt (22) suggest that a bacterial system is not involved in tunicate luminescence. Mackie and Bone (55), on the other hand, have published a detailed ultrastructural study of the light organ of a pyrosome, which suggests that bacteria are indeed present; they propose that bacteria are responsible for the light emission. In fact, bacterial luciferase activity has been demonstrated recently in extracts of both salps (J. C. Makemson, M. Haygood, J. Dunlap, and J. W. Hastings, unpublished data) and pyrosomes (G. Leisman, D. Cohn, and K. H. Nealson, unpublished data). In the latter study, the luminescence was not due to external luminous bacteria. Although such adventitious luminous bacteria were present, their luciferase activity was distinguishable, on the basis of decay kinetics (slow), from that of the pyrosome extracts (fast).

The possibility that bacteria provide the source of light for tunicates is of special interest in connection with the manner by which the luminescence is controlled. Pyrosomes respond to external stimuli, producing rapid (less than 0.1-s) flashes of light. The nature of the control system is of great interest, as it relates to the degree of cellular integration of the symbionts. For example, does the host actually control the biochemical flashing of the bacterial luciferase or merely mediate a constant bacterial glow by other methods? In other symbiotic systems, fish and squid, for example, the light is controlled by means of shutters or chromatophores. No such structures are obvious in the pyrosomes, and the possibility that the biochemistry of light emission of these endosymbionts is directly controlled by the host is considered by Mackie and Bone (55).

## FUNCTIONS OF BIOLUMINESCENCE

### Vestigial or Nonfunctional Hypotheses

Ten years ago the suggestion that the luminescence system in bacteria is a vestigial one, having no present day function, was still being discussed (35). Knowledge of the elaborate mechanisms controlling the biosynthesis and activity of luminescence now makes such a view unlikely, if not altogether untenable (40). Likewise, the possibility that luminescence is the obligatory concomitant of some other functional system has been discarded.

### Biochemical Functions

Ten years ago the possibility that the emission of light might relate to a biochemical (as distinct from a biological) function was put forward and discussed in detail (35). A specific suggestion

made then was that a bioluminescence system could allow a cell to carry out "photochemistry in the dark," utilizing excited states produced biochemically, possibly in the form of singlet oxygen. According to this hypothesis, photochemistry would be an alternative to light emission, so that the functionally important condition need not be bioluminescence; in addition, the light emitted would itself be nonfunctional. No support for this idea has appeared, but to our knowledge no good tests or experiments have been carried out to examine it.

Another possible biochemical function is that the light-emitting system can function as an alternate pathway for electron flow, analogous to the alternate carriers already known in *E. coli* (27, 81), *Klebsiella* (30), and *Beneckeia natrie-gens* (54). In luminous bacteria it is known that luciferase functions in vivo at very low oxygen concentrations (32, 34), even at concentrations so low that electron flow via the cytochromes is essentially stopped. Under these conditions, luminous bacteria may have an energetic advantage because the electron flow via luciferase would allow for the reoxidation of reduced coenzymes and some adenosine triphosphate formation. Although the adenosine triphosphate production associated with cytochrome electron flow is lost, that associated with the flow from pyridine nucleotide to flavin is not, so that the phosphate-electron ratio might be changed from 3 to 1, a situation greatly favorable to fermentative metabolism. This mode of metabolism might represent a major advantage under low-oxygen conditions with nonfermentable carbon sources. Indeed, this might explain why bright strains survive low-oxygen conditions better than the dark K variants do (Nealson, Ph.D. thesis).

From an ecological point of view, the ability to function well under microaerophilic conditions may be of significance for both the symbiotic and the saprophytic luminous bacteria. Within light organs, where symbiotic bacteria are cultured, the oxygen concentration may be poised at a low level. In the saprophytic state, luminous bacteria live as heterotrophs associated with organic matter. This ecological niche may also involve a low oxygen tension, so a physiological adaptation to respire under such conditions may be advantageous ecologically.

### Biological Functions

Most authors favor the idea that it is the emitted light that is of functional importance and that its perception by some other organism(s) initiates phenomena which ultimately serve to the advantage of the light-emitting bacteria. It seems unlikely that intraspecific effects

occur, since the behavior of luminous bacteria is unaffected by visible light (32).

**Light organ luminescence.** It was known as early as 1921 that light organs of certain fish harbor luminous bacteria as the source of their luminescence (31); bacteria from *Monocentris* were cultured a few years later (99). The function of the light is known in many of these cases and relates to the behavior of the host, not the bacteria (see above). The host possesses the capability to control the light via shutters and other mechanisms. In return for light production, the bacteria are provided with nutrients, oxygen, and a protected niche. Descriptions of such light organs and the functions of light emission in these cases are found elsewhere (4, 5, 28, 32, 40, 45, 50, 65, 91).

**Dispersion and propagation.** Luminous bacteria apparently flourish and emit light in numerous niches outside specific light organs, some of which are in association with other organisms (parasitic and commensal) and some of which are not (saprophytic). One possible view is that these bacteria are opportunistic escapees or an inevitable overflow from the light organ niche and that their light emission in these other situations has no strong positive selective advantage.

Another view, which we favor, is that there is positive selection and that the selection mechanism may relate to the dispersion and propagation of the bacteria. If these bacteria are truly marine enterobacteria (7), then it may be postulated that attraction of organisms that will ingest them is advantageous. The facts that luminous bacteria are abundant in fish intestines (85; Ruby, Ph.D. thesis) and that marked (mutant) strains of luminous bacteria survive passage through the gut tracts of fish (Ruby, Ph.D. thesis) support this view. Luminous bacteria growing on a substrate, whether it is a parasitized crustacean, the surface of a dead fish or squid, or a fecal pellet, could produce sufficient light to attract organisms to feed on the material, thus enhancing the propagation of the bacteria (40). In fact, based on analyses of fish gut contents, Robinson and Morin (Abstr. Annu. Meet. West. Soc. Natural. 1977) have argued for such an hypothesis, viewing the bacteria in the fish guts as an aid to chitin digestion and the luminescence of fecal pellets as a selective advantage to the bacteria for their own propagation.

For *Beneckeia* species, which are not known to occur as specific symbionts, this free-living mode may represent a major and exhaustively exploited way of life. For *Photobacterium* species, it may represent a light organ-independent mode for which the property of luminescence may nevertheless still have a positive selective value.

For the postulate to be valid, the light must be seen. For the light to be seen there must be more than a single bacterium, although the number is difficult to specify, given the complexities of the optics (absorption, reflection, solid angle subtended, etc.) and the distribution of the bacteria (size and shape of source) in any given ecological situation. Bright, fully induced bacteria emit between  $10^3$  and  $10^4$  quanta/s per cell (26). Photoreceptors can be sensitive to as few as 5 to 10 quanta, perhaps fewer, but this means quanta impinging on the photoreceptor after all losses have been suffered. Moreover, photoreceptors adapt readily and are more sensitive to changes in the light intensity than to absolute levels, so the bacterial light emission, being continuous, must either be modulated by some mechanism or be relatively bright in comparison with the surrounding environment to be significant ecologically. With all this in mind, it seems likely that a flux of  $10^8$  quanta/s per  $\text{mm}^2$  of emitting surface is the minimum needed to be seen. This would require  $10^4$  or  $10^5$  cells; if this number corresponded to  $1 \text{ mm}^3$  of bacterial culture, it would represent a cell density of  $10^7$  or  $10^8$  cells per ml, which is just adequate for autoinduction. Both light organ cultures and saprophytic growths are two or more orders of magnitude more dense and thus brighter than the minimum value discussed above (39, 86).

#### PERSPECTIVES: LUMINOUS AND NONLUMINOUS BACTERIA

It now seems clear that the versatility of the luminous species is not limited to alternate uses of light. These bacteria are also capable of successfully exploiting habitats where there is apparently no positive selection for the ability to emit light. One way to compete with nonluminous forms, assuming that the energetic drain due to bioluminescence is a disadvantage, is to have the capacity to block the synthesis of the light-emitting system.

Thus, the phenomenon of autoinduction can be viewed as a mechanism that allows for physiological adaptation to alternate environments. The environment that is favorable for autoinduction is relatively nonspecific; it requires only that the bacteria grow in a confined situation so that the autoinducer accumulates. The fact that autoinduction occurs in both *Photobacterium* and *Beneckeia* species suggests that light emission is functionally important and has a selective advantage in situations other than light organs. Under conditions of low numbers of bacteria, when even bright bacteria would not be seen anyway, the lack of autoinduction insures that the cells are repressed for luminescence.

Why do nonluminous species of *Beneckeia* produce autoinducer that is active in inducing the luminous strains? Are they inducing the luminous strains under conditions where it will not confer selective advantage, so that the nonluminous strains overgrow the luminous ones? Or is it a bacterial mutualism where the two species collaborate in the production of luminescence that profits both? There are surely numerous variations on these (hypothetical) scenarios, and it is not yet possible to arrive at any favored hypothesis for any of the luminous species.

Catabolite repression represents still another specific mechanism whereby control over the synthesis of the bioluminescence system can be exerted. The ecological significance of the distinctions between the *Beneckeia* and *Photobacterium* species in this regard is not clear, although it may relate to the fact that the light organ symbionts (*Photobacterium* species) receive nutrients from the host via the bloodstream, which may be high in glucose. Knowledge concerning the blood sugar(s) in the host fish would be valuable. Repression of luminescence is obviously not consistent with light organ function, but it may be important in the guts of fish, where it could be mediated by the product of chitin digestion (*N*-acetylglucosamine).

More permanent control of luminescence may be achieved via the reversible transformation to genetically stable dark forms, the K variants. Under conditions where luminescence has no selective advantage, the K variants are presumably selected for, and conversely, the bright revertants are selected for when luminescence is advantageous. Thus, the propagation of either form provides a potential inoculum for the other. However, the factors that select for or against luminescence are not known. If the ecology of the luminous bacteria is to be understood, the importance of the K variants must be established, as well as the factors that lead to their formation and reversion. Are the naturally occurring dark bacteria that are taxonomically identified as *Beneckeia* and *Photobacterium* species merely naturally occurring K variants? Does the differential sensitivity to anaerobic conditions in the two forms provide a mechanism whereby the light organ can periodically purge itself of any dim K variants? Is there a selective advantage for the dark variants over the wild-type bright variants under any conditions? Does the capacity to form stable dark variants allow for life in some niche outside the symbiotic mode, where luminescence is selected against? Clearly, these and other such questions are central to the understanding of the ecology of the

luminescent bacteria.

There are other relevant questions. What is the cellular energetic commitment to bacterial light emission? Are some of the luminous bacteria low-nutrient forms? Can they grow as free-living forms in the ocean? What is the relationship between the various symbiotic partnerships and the population densities of luminous bacteria? Have luminous bacteria established themselves in true endosymbiotic relationships in addition to the well-known extracellular ones? What is the abundance, distribution, and significance of luminous bacteria in the gut tracts of marine organisms? What are the physiological mechanisms involved in the establishment, maintenance, and control of the various symbioses?

Although the questions are many and the firmly supported models are few, information concerning the biochemistry, physiology, and distribution of the luminous bacteria continues to accumulate. As the data appear, many of the questions posed above will be answered, and an understanding of the ecology of these abundant, widespread, and spectacular bacteria will be possible.

#### ACKNOWLEDGMENTS

Although we are responsible for the ideas and interpretations presented in this review, we were greatly aided in their formulation and alteration by discussions with J. Morin, M. Shilo, P. Baumann, J. Reichelt, R. Rosenblatt, and S. Ulitzur. In addition, unpublished data and preprints were supplied by A. Eberhard, M. Shilo, E. G. Ruby, E. P. Greenberg, G. Leisman, D. Cohn, B. Tebo, G. Poinar, S. Ulitzur, D. Karl, K. Kopecky, P. Dunlap, J. Morin, and J. Baross. Without this assistance, it would have been impossible to do a complete job on the review, and we are most grateful.

We acknowledge the support of National Science Foundation grants PCM74-14788 (to K.H.N.) and PCM77-19917 (to J.W.H.).

#### ADDENDUM IN PROOF

The compound responsible for the autoinducer activity from *P. fischeri* has now been purified and identified as *N*-(3-oxohexanoyl)-3-aminodihydro-2(3*H*)-furanone (*N*- $\beta$ -keto-hexanoyl-homoserine lactone) (A. Eberhard, personal communication).

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#### ADDENDUM IN PROOF

The compound responsible for the autoinducer activity from *P. fischeri* has now been purified and identified as *N*-(3-oxohexanoyl)-3-aminodihydro-2(3*H*)-furanone (*N*- $\beta$ -keto-hexanoyl-homoserine lactone) (A. Eberhard, personal communication).