the left and right buccal ganglia. To study the relation between neural excitation and propagation of the gland action potential, we utilized a preparation in which the paired buccal ganglia were left attached to one side of the salivary gland. Figure 2B shows records from an experiment in which activity in one of the effector neurons was monitored with an intracellular microelectrode while activity in the gland was monitored optically. Firing of the effector neuron evoked "optical spikes" in the gland reflecting the propagation of gland action potentials that followed the neuronal activity at a constant latency. The arrow indicates a large optical spike that preceded firing in the effector neuron and that probably arose from another excitatory input. Since it was not possible to monitor activity in both effector neurons simultaneously, we cannot be certain that the larger optical spike was the result of activity in the other effector neuron. However, it is clear that different sources of excitatory inputs to the gland can evoke optical spikes of significantly differing amplitudes. Furthermore, it is reasonable to assume that the differences in the optical signal reflect differences in the degree to which a regenerative spike propagates through the gland. A photodiode array, monitoring simultaneously the optical signals from a large number of discrete regions of the gland, could be used to study conduction pathways in this and other electrical syncytia.

These results demonstrate the feasibility of optically monitoring membrane potential in an exocrine gland. We believe that it will be possible to use this approach in electrophysiological studies of other exocrine tissues. Further, we hope that this technique may prove a powerful tool for the study of excitation-secretion coupling in endocrine glands.

Note added in proof: Ross and Reichardt (*17*) found a similar triphasic wavelength dependence of the optical signals from rat superior cervical ganglion cells with the merocyanine-oxazolone and merocyanine-rhodanine dyes.

D. M. SENSEMAN
Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104 and *Department of Physiology and Pharmacology, School of Dental Medicine, University of Pennsylvania, Philadelphia 19104*

B. M. SALZBERG
Department of Physiology and Pharmacology, School of Dental Medicine and *Institute of Neurological Sciences, University of Pennsylvania, Philadelphia 19104*

References and Notes

1. S. B. Kater, *Am. Zool.* **14**, 1017 (1974); I. Atwater and H. P. Meissner, *J. Physiol. (London)* **247**, 56P (1975); P. S. Taraskevich and W. W. Douglas, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4064 (1977); B. Dufy, J.-D. Vincent, H. Fleury, P. DuPasquier, D. Gourdji, A. Tixier-Vidal, *Science* **204**, 509 (1979); E. K. Matthews and J. Saffran, *J. Physiol. (London)* **189**, 149 (1967).
2. W. W. Douglas and P. S. Taraskevich, *J. Physiol. (London)* **285**, 171 (1978).
3. O. H. Peterson, *Physiol. Rev.* **56**, 535 (1976).
4. T. Tasaki, A. Watanabe, R. Sandlin, L. Carnay, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 883 (1968); L. B. Cohen, B. M. Salzberg, H. V. Davila, W. N. Ross, D. Landowne, A. S. Waggoner, C. H. Wang, *J. Membr. Biol.* **19**, 1 (1974); W. N. Ross, B. M. Salzberg, L. B. Cohen, H. V. Davila, *Biophys. J.* **14**, 983 (1974).
5. A. Grinvald, B. M. Salzberg, L. B. Cohen, *Nature (London)* **268**, 140 (1977).
6. B. M. Salzberg, A. Grinvald, L. B. Cohen, H. V. Davila, W. N. Ross, *J. Neurophysiol.* **40**, 1281 (1977).
7. G. Salama and M. Morad, *Science* **191**, 485 (1976); M. Morad and G. Salama, *J. Physiol. (London)* **292**, 267 (1979).
8. H. Oetliker, S. M. Baylor, W. K. Chandler, *Nature (London)* **257**, 693 (1975); F. Bezanilla and P. Horowicz, *J. Physiol. (London)* **246**, 709 (1975); J. Vergara and F. Bezanilla, *Biophys. J.* **17**, 5 (Abstr.) (1977); _____, B. M. Salzberg, *J. Gen. Physiol.* **72**, 775 (1978).
9. L. B. Cohen and B. M. Salzberg, *Rev. Physiol. Biochem. Pharmacol.* **83**, 35 (1978).
10. A. S. Waggoner, *Annu. Rev. Biophys. Bioeng.* **8**, 47 (1979).
11. A potential-sensitive dye was used by P. F. Baker and T. J. Rink [*J. Physiol. (London)* **253**, 593 (1975)] to confirm that maintained exposure to high K^+ levels produces a stable depolarization of thin slices of bovine adrenal medulla.
12. B. M. Salzberg and D. M. Senseman, *Neurosci. Abstr.* **5**, 260 (1980).
13. S. B. Kater, J. R. Rued, A. D. Murphy, *J. Exp. Biol.* **72**, 77 (1978); *ibid.*, p. 91.
14. S. B. Kater, C. Heyer, J. P. Hegmann, *Z. Vgl. Physiol.* **74**, 127 (1971).
15. L. B. Cohen, A. Grinvald, K. Kamino, S. Lesher, B. M. Salzberg, unpublished observations.
16. To obtain a direct-coupled optical measurement, it was necessary to use a digital sample-and-hold circuit to provide a sufficiently stable and accurate d-c offset potential. This was implemented with 12-bit A-to-D and D-to-A converters in a simple back-to-front configuration.
17. W. N. Ross and L. F. Reichardt, *J. Membr. Biol.* **48**, 343 (1979).
18. We thank J. Brand, L. B. Cohen, R. D. Fish, and R. K. Orkand for their critical reading of the manuscript. Supported in part by National Science Foundation grant BNS 770525, National Institute of Dental Research grants DE-05271 and DE-05536, and Biomedical Research Support grant RR-05337-17.

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Bacterial Origin of Luminescence in Marine Animals

Abstract. *Bacterial luciferase activity was detected in light organ extracts of squids, fishes, and pyrosomes, suggesting that these systems are derived from bacteria-animal symbioses. In none of these cases was it possible to culture luminous bacteria. Analyses of the decay kinetics show that the luciferases from the squid, ceratioid, and pyrosome light organs are all similar to bacterial luciferases from the genus* Photobacterium, *while those from the anomalopid light organs are different.*

Bioluminescence is common in marine organisms; estimates are that 90 percent or more of midwater animals are capable of light emission (*1*, *2*). In bacteria and protists, the entire organism is usually luminous, but as structural complexity increases there is a tendency for light emission to be restricted to specialized organs. Such bioluminescence in marine fishes and squids may be an intrinsic property of the animal tissue in photophores, or may emanate from symbiotic bacteria harbored in specialized light organs (*3*). The symbioses exhibit species specificity; members of a given fish or squid family always have the same species of bacteria associated with them (*4*). Of the two recognized genera of marine luminous bacteria, *Beneckea* and *Photobacterium*, only species of *Photobacterium* have been found as light organ symbionts. However, in some cases the distinction between symbiotic and intrinsic light emission is not easily made; bacteria-like bodies (bacteroids) are present in some light organs, but no luminous bacteria have been cultured from them (*2*, *3*, *5*-*8*). We describe here enzyme methods that can be used in such cases to establish the presence and identity of bacterial symbionts.

Bacterial luciferase, a mixed-function oxidase, catalyzes light emission via the reaction shown in Fig. 1. The enzyme is found only in prokaryotes, and thus is not found in nonbacterial luminescent systems. An unusual feature of the enzyme is its slow rate of turnover, which is dependent on the aldehyde used in the in vitro reaction (*9*). If dodecanal is the aldehyde used, luciferases isolated from the two bacterial genera are different in their kinetic properties; those from *Beneckea* exhibit slow decay kinetics, while those from *Photobacterium* give fast decay (Fig. 1, inset). On the basis of this difference, luciferase decay kinetics have been used to identify the bacterial genus from which the luciferase was extracted (*10*).

In our study, light organ extracts of several fishes, squids, and tunicates were examined (Table 1). Bacterial luciferases were detected and kinetically characterized, even though the bacterial symbionts could not be cultured (*11*).

Extracts from the light organs of six specimens of the squid, *Heteroteuthis hawaiiensis*, were tested. All were positive for bacterial luciferase and showed fast decay kinetics, characteristic of the genus *Photobacterium*.

Extracts of two specimens of *Pyrosoma* sp. (Tunicata, Pyrosomida) were

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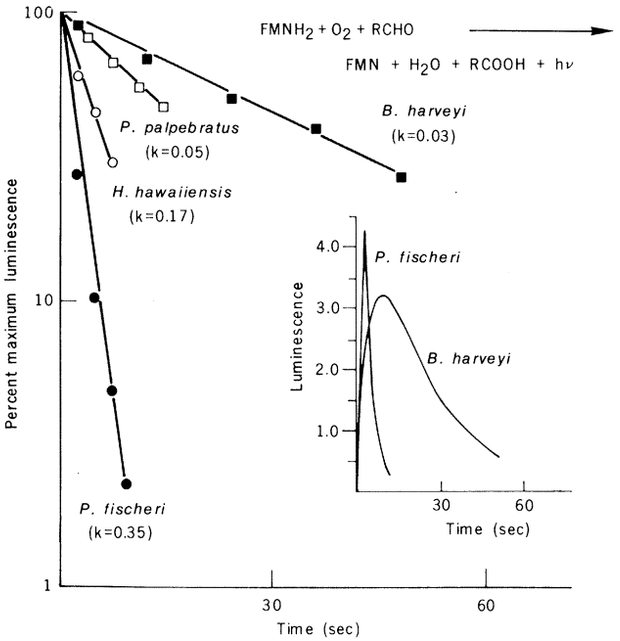

Fig. 1. Decay kinetics of luciferases extracted from bacteria or light organs of luminous animals. Two bacterial controls (*Beneckea harveyi*, —■—; *Photobacterium fischeri*, —●—) and two light organ extracts (*Photoblepharon palpebratus*, —□—; *Heteroteuthis hawaiiensis*, —○—) are shown. The first-order decay constants (k) are expressed as reciprocal seconds. Decay rates of luciferases from various *Beneckea* species and strains range from 0.01 to 0.05 per second, while those of the *Photobacterium* species range from 0.16 to 0.35 per second (*18*). Thus the slowest of the fast types and the fastest of the slow types show that, even in the worst case, the distinction between fast and slow is unequivocal. Typical assays (inset) illustrate the difference between fast and slow decay types as determined in laboratory experiments. The luciferase assay was performed by adding 10 to 100 μl of the light organ extract in lysis buffer (10 m*M* EDTA, 1 m*M* dithiothreitol, *p*H 7.0) to a vial containing 1 ml of buffer (100 m*M* potassium phosphate, *p*H 7.0), 1 ml of FMN (5×10^{-5} *M*), and 10 μl of a 0.1 percent suspension of dodecanal in water. The reaction was initiated by the addition of several grains of sodium dithionite (just enough to change the color of the solution from yellow to clear). The light was measured in an ATP photometer (SAI model 2000) and recorded on a strip chart recorder. Extracts of known luminous bacteria were used as controls (*9*).

Table 1. Light organ analyses. Squid, angler fish (except *Oneirodes* sp.), and pyrosomes were collected in midwater trawls from the R.V. *Kana Keoki* in June 1978 and September 1979 off leeward Oahu. *Oneirodes* sp. were collected in midwater trawls from the R.V. *Velero IV* in San Nicholas basin off the southern California coastline in July 1978. *Kryptophanaron alfredi* was collected during dives off Grand Cayman Island in February 1978. *Photoblepharon palpebratus* and *Anomalops katoptron* were provided by J. McCosker, Steinhart Aquarium, San Francisco. The animals or light organ surfaces were first washed with sterile seawater or ethanol; the organ, in a sterile petri dish, was then cut open with an ethanol-sterilized scalpel. Samples were diluted and spread on plates containing seawater nutrient agar [SWC (*9*)] or other solid media (no luminous colonies were ever cultured). To extract the bacterial luciferase, lysis buffer (10 m*M* EDTA, 1 m*M* dithiothreitol, *p*H 7.0) was added directly to a portion of the light organ in a test tube or to a seawater homogenate of the light organ. Pyrosomes were first homogenized; some portions of this homogenate were spread on plates containing SWC to detect viable bacteria, while other portions were sonicated to release the bacterial luciferase. In all cases, the luciferases were assayed as described in the legend of Fig. 1. Abbreviation: N.R., no report.

Family	Species	Number tested	Decay kinetics	Decay constant (sec^{-1})	Bacteroids
	Squid				
Sepiolidae	*Heteroteuthis hawaiiensis*	6	Fast	0.17	+
	Pyrosomes (Tunicata)				
Pyrosomida	*Pyrosoma* sp.	2	Fast	0.16	+
	Angler fish (Ceratioida)				
Ceratiidae	*Cryptopsarus couesi*	4	Fast	0.29	N.R.
	Ceratias holboelli	4	Fast	0.38	N.R.
Melanocetidae	*Melanocetus johnsoni*	3	Fast	0.25	N.R.*
Oneirodidae	*Oneirodes* sp.	2	Fast	0.36	N.R.*
	Flashlight fish				
Anomalopidae	*Photoblepharon palpebratus*	1	Slow	0.05	+
	Anomalops katoptron	1	Slow	0.03	+
	Kryptophanaron alfredi	2	Slow	0.03	+
	Bacterial controls				
	Beneckea harveyi		Slow	0.03	
	Photobacterium fischeri		Fast	0.35	

*Bacteroids observed in species other than those reported here: *Malanocetus murrayi* (*12*) and *Oneirodes acanthias* (*7*).

also examined and yielded luciferase activity with fast decay kinetics. Because of the small size of the light organ and the gelatinous nature of the organism, it was not possible to sample the light organ alone. The gelatinous coating and accompanying intercellular fluid contained high numbers of bacteria (about 10^6 per milliliter), but none were luminous.

Four species of ceratioids (angler fish) were examined for bacterial luciferase activity in their luminous esca (bait). All had bacterial luciferase activity with fast decay kinetics. Ceratiid species have, in addition to the esca, caudal light organs [caruncles (*2*)]. The caruncles of seven specimens were examined; six had detectable luciferase activity, and all of these exhibited fast decay kinetics.

Light organ extracts of all three genera in the family Anomalopidae (flashlight fishes) contained bacterial luciferase, but unlike the pyrosomes, angler fishes, and squids, the luciferases exhibited slow decay kinetics. Luciferase activity (*7*) and bacteroids (*5–7*) have been reported from two of these genera, *Anomalops* and *Photoblepharon*.

The presence of luciferase with slow decay kinetics in the light organs of the Anomalopidae is unusual, since all previously reported symbiotic bacteria have luciferases with fast kinetics. Several explanations might account for the slow kinetics. First, a *Beneckea* species may be the symbiont in the light organ. Second, a new *Photobacterium* species, one with a slow enzyme, may be present in the light organs. If so, it is the first member of the genus *Photobacterium* with such a luciferase (*10*). Third, the bacterial symbionts of the Anomalopidae may be an entirely different group of luminous bacteria, possibly a new genus. In this regard, it should be noted that the taxonomy and ecology of the luminous bacteria are only now beginning to be understood, and it is thus not unreasonable to expect that new species or even new genera will be found as these studies proceed. However, until the symbionts of these organisms can be grown in culture, it will be difficult to determine which hypothesis is correct.

The presence of bacterial luciferase in the ceratioid escas is consistent with the reports of others (*5, 7, 12*) who, on the basis of ultrastructural analyses, postulated that angler fishes are symbiotically luminous, even though no symbionts could be cultured outside the host. Similarly, the detection of bacterial luciferase activity in the extracts of the ceratiid caruncles is in agreement with the report (*13*) that these organs contain symbiotic luminous bacteria.

The detection of bacterial luciferase in the luminous squid, *Heteroteuthis hawaiiensis*, represents a controversial case in that another species in this genus (*H. dispar*) has been reported to lack symbiotic bacteria (*14*). Ultrastructural examination of the light organs of *H. hawaiiensis* has revealed bacterialike bodies 2 to 3 μm in diameter, yet typical bacteroids were not seen (*15*). On the basis of both ultrastructural data and the inability to cultivate bacteria from the light organ, Dilly and Herring (*14*) suggested that bacteria were not present in the related *H. dispar*; however, they did report the presence of large "bacterialike particles."

The bacterial origin of luminescence in pyrosomes has long been debated (*3*). However, on the basis of ultrastructural analyses of the light organs, it was proposed (*16*) that pyrosomes contain symbiotic luminous bacteria. Our results support the idea that the light is of bacterial origin. The presence of intracellular symbionts, as proposed (*16*), would represent yet another level of integration that the bacterial symbionts have undergone (*17*).

The luminous bacteria–marine animal symbioses represent a continuum of associations, ranging from easily cultured, facultative, extracellular symbionts, to extracellular bacteroid symbionts, to intracellular bacteroid symbionts. This continuum may reflect a gradual integration of the several hosts with the luminous bacteria and could provide a series of model systems for the study of the evolution of symbiosis. The bacterial luciferase assay is a valuable probe in these studies.

GARY LEISMAN
DANIEL H. COHN
KENNETH H. NEALSON
Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, California 92093

References and Notes

1. W. M. Beebe, *Zoologica (Stuttgart)* **22**, 197 (1937).
2. P. J. Herring and J. G. Morin, in *Bioluminescence in Action*, P. J. Herring, Ed. (Academic Press, New York, 1978), p. 273.
3. E. N. Harvey, *Bioluminescence* (Academic Press, New York, 1952).
4. K. H. Nealson, *Trends Biochem. Sci.* **4**, 105 (1979); _____ and J. W. Hastings, *Microbiol. Rev.* **43**, 496 (1979).
5. J. M. Bassot, in *Bioluminescence in Progress*, F. H. Johnson and Y. Haneda, Eds. (Princeton Univ. Press, Princeton, 1966), p. 557.
6. M. Kessel, *Cytobiologia* **15**, 145 (1977).
7. W. T. O'Day, *Contributions to Science from the Los Angeles County Museum No. 255* (1974).
8. Y. Haneda and F. I. Tsuji, *Science* **173**, 143 (1971).
9. The substrates of the reaction are reduced flavin mononucleotide ($FMNH_2$), oxygen, and one of several long-chain aliphatic aldehydes (RCHO). Oxygen oxidizes the other two substrates in a reaction that yields visible light with a maximum at ~ 490 nm. There is a long-lived enzyme-substrate intermediate that has a half-time of decay on the order of seconds, and any excess $FMNH_2$ autooxidizes to FMN in milliseconds. Thus each enzyme molecule turns over only once during the assay, producing a characteristic kinetic profile (Fig. 1, inset) [K. H. Nealson, *Methods Enzymol.* **57**, 153 (1978)].
10. Luciferase decay kinetics have been examined in extracts of several thousand luminous bacterial isolates independently identified by numerical taxonomy. With no exceptions, members of the genus *Photobacterium* had fast decay kinetics and luminous *Beneckea* isolates had slow decay kinetics [E. G. Ruby, thesis, University of California, San Diego (1977)].
11. In some cases, extensive attempts were made to culture the bacteria using as many as 150 different media. No luminous colonies were ever obtained. In every case some dark colonies did grow on the plates; these were tested for bacterial luciferase activity and all were negative.
12. W. H. Hulet and G. Musil, *Copeia* **3**, 506 (1968); K. Hansen and P. J. Herring, *J. Zool. Proc. Zool. Soc. London* **182**, 103 (1977).
13. Unpublished data cited in (*2*), p. 324.
14. P. N. Dilly and P. J. Herring, *J. Zool. Proc. Zool. Soc. London* **186**, 47 (1978).
15. R. E. Young, personal communication.
16. G. O. Mackie and Q. Bone, *Proc. R. Soc. London Ser. B* **202**, 483 (1978).
17. Pyrosomes flash in response to a variety of stimuli [see (*3*, *7*)]. Since luminous bacteria in culture emit light continuously and those in the transparent pyrosome emit light only upon stimulation, the control of the bacterial light-emitting system may reside in the pyrosome host; the mechanism by which this might occur is unclear.
18. K. H. Nealson, unpublished data.
19. We thank R. E. Young and J. J. Childress for the opportunity to participate in cruises aboard the R.V. *Kana Keoki* (FIDO XII and XIV) and the R.V. *Velero IV*. We thank the crew of the *Cayman Diver* for assistance in collecting specimens. J. McCosker (Steinhart Aquarium) supplied some specimens. T. W. Pietsch identified the ceratioids. M. Haygood, R. Rosson, and B. Tebo helped with specimen collection and enzyme assays. R. E. Young, J. G. Morin, and L. Margulis provided helpful comments on the manuscript. Supported by NSF grant PCM 74-14788.

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Purinergic Receptors: Photoaffinity Analog of Adenosine Triphosphate Is a Specific Adenosine Triphosphate Antagonist

Abstract. *Arylazido aminopropionyl adenosine triphosphate ($ANAPP_3$), a photoaffinity label, antagonized specifically adenine nucleotide–induced contractions of the guinea pig vas deferens. Irradiation of tissues with visible light in the presence of $ANAPP_3$ resulted in an irreversible antagonism, which was prevented when adenosine triphosphate was present. In the absence of light, the antagonism was reversible and may have resulted from an autoinhibition phenomenon. Responses to acetylcholine, histamine, norepinephrine, and potassium chloride were not affected by $ANAPP_3$.*

There is considerable evidence that many visceral organs of vertebrates are innervated by motor neurons, termed purinergic nerves by Burnstock (*1*), that use adenosine triphosphate (ATP) as a neurotransmitter. Many criteria to establish ATP as a transmitter have been met (*2*). However, a specific pharmacological antagonist to ATP-induced responses (*3*), which would unequivocally demonstrate the existence of purinergic receptors (*4*), has not been available to satisfy the criterion that neurally released ATP is functionally important as a transmitter. One compound, 2,2′-pyridylisatogen, showed promise initially as a selective ATP antagonist (*5*). However, more recent evidence (*6*) indicates that pyridylisatogen has undesirable nonspecific effects.

Arylazido aminopropionyl ATP (3′-*O*-{3-[*N*-(4-azido-2-nitrophenyl)amino]propionyl}adenosine 5′-triphosphate), or $ANAPP_3$, was shown to be a photoaffinity label of rabbit skeletal muscle myosin and F_1-mitochondrial adenosinetriphosphatases (*7*). Being a derivative of ATP, $ANAPP_3$ is, without light activation, a substrate for these enzymes (*7*). Photoactivation with visible light induces a specific covalent attachment to the adenosinetriphosphatase with a resultant decrease in enzyme activity. We reasoned that the photoaffinity characteristics of $ANAPP_3$ might be applied to intact tissues to study the interactions of adenine nucleotides with smooth muscle.

Vasa deferentia from adult guinea pigs were placed in separate water-jacketed glass organ chambers (*8*). $ANAPP_3$ was synthesized by the method of Jeng and co-workers (*7*). The effects of $ANAPP_3$ on concentration-response relationships were evaluated in "light" and "dark" experiments by the following protocols. In light experiments, one tissue of a pair was exposed, in the absence of light, to $ANAPP_3$ for 3 minutes. The $ANAPP_3$ caused an immediate, ATP-like contraction (*9*), which quickly subsided. The tissue was then irradiated in the organ bath for 30 minutes with a tungsten-halogen projector lamp (model DVY, 650 W, 3400 K) (*7*), the filament of which was approximately 10 cm from the tissue. In the presence, but not in the absence, of $ANAPP_3$, irradiation induced a small sustained contraction, at times with cyclic spontaneous contractions superimposed. At the end of photoactivation, fresh bathing medium lacking $ANAPP_3$ was introduced. The remainder of the experiment was begun 4 minutes later and conducted in the dark. In dark experiments, one tissue of a pair was incubated in $ANAPP_3$ for 33 minutes in the

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