Meetings

Bioluminescence

The emission of light without perceptible heat occurs in a great variety of living organisms including bacteria, fungi, many marine animals, fireflies, and others. Numerous aspects, such as the origin, evolution, and mechanisms of this phenomenon known as bioluminescence still remain largely unknown. New discoveries and recent research in this field were the topics of discussion at a Luminescence Conference, held in Japan (12–16 September 1965) under the auspices of the U.S.-Japan Cooperative Science Program.

In the opening paper, J. R. Totter proposed a reaction sequence for intermediary steps leading to light emission of alkylbiacridylium compounds and the phthalazinediones, with special reference to lucigenine and luminol, respectively. Although only the starting compounds and some of the end products are known with certainty, the influences of oxygen and other factors on the kinetics of the chemiluminescence indicate reduction steps in the overall oxidative process, and permit the derivation of quantitative expressions which, with the appropriate rate and equilibrium constants, account for the course of light intensity.

The conveniently useful application of the chemiluminescence of luminol as a means of calibrating phototube responses in terms of absolute units of light emission was considered, by Lee, Wesley, Ferguson, and Seliger, and by Hastings and Reynolds. The results of their methods agreed with data obtained by quite different methods. The general importance of reporting data on chemi- and bioluminescence in absolute units was stressed.

The chemiluminescence of indole compounds, recently discovered by Cormier and Totter, is a phenomenon of special interest for two reasons—the active substrate, or "luciferin," of the famous *Cypridina* (ostracod crustacean) system is an indole derivative (Shimomura, Goto, and Hirata), and luciferins in other systems, including those of the sea pansy *Renilla* and the acorn worm *Balanoglossus* (Cormier and colleagues) are also indole derivatives. Synthesis of some 25 new indole compounds, of interest with reference to the structure of *Cypridina* luciferin, was reported by Stachel, Taylor, Shimomura, and Johnson. An investigation of the chemiluminescence and fluorescence of these compounds, and also of *Cypridina* luciferin in dimethylsulfoxide, was reported (Johnson, Stachel, Taylor, and Shimomura). Data on the chemiluminescent oxidation of a variety of other indole derivatives in aqueous solution, as well as in dimethylsulfoxide, were contributed by Sugiyama, Akutugawa, Gasha, and Saiga.

The long-standing difficult problem of the chemical structure of *Cypridina* luciferin (a problem which may be said to have begun some 50 years ago when the late E. Newton Harvey was inspired to a life-long interest while studying this system in Japan) was finally brought to a definitive solution by Kishi, Goto, Hirata, Shimomura, and Johnson.

That bioluminescence systems can be used as model enzyme systems for analyzing the action of various factors of biological importance was demonstrated by Chase's analysis of the urea inhibition of *Cypridina* luciferase. The potential usefulness of the luminescence system of *Cypridina*, luminous bacteria, and fungi as a sensitive means of detecting contamination of the atmosphere in space vehicles by traces of jet fuels was noted by Sie, Thanos, and Jordon.

The crustacean *Cypridina* system was again considered in regard to the virtually unique "cross-reaction" between its specific substrate and enzyme (luciferin and luciferase) components and those of a biologically different type of organism, namely certain fishes (*Apogon* and *Parapriacanthus*). From immunological reactions Tsuji and Haneda showed the luciferase components from the two types of organisms differ. On the other hand, luciferin in photogenic organs of such fish (*Parapriacanthus*) might actually be acquired by feeding on *Cypridina* (Haneda, Johnson, and Shimomura).

The bio- and physical chemistry, and reaction kinetics of int ediary compounds involved in the luminescence systems of fungi, the sea pansy, acorn worm, and firefly were discussed in great detail.

Methods of obtaining, on a fairly large scale, luminescent mycelia in submerged cultures have been developed by Wassink and Kuwabara. Such cultures are important in biochemical purification procedures. Large-scale cultivation of luminous bacteria, and evidence of a pteridine requirement for production of the luminescence system in these organisms, was described by Nakamura.

In the sea pansy (*Renilla*) system, light emission requires activation of the luciferin by an enzyme with the cofactor 3′,5′-diphosphoadenosine, or prior activation by heat (Cormier, Hori, and Kreiss). Cormier and his associates Kreiss and Pritchard also discussed the luminescence system of the acorn worm (*Balanoglossus*) which is unusual in requiring H_2O_2 but not molecular oxygen. Evidence was presented that this is a typical peroxidase system, and analysis of the kinetics led the authors to propose a new mechanism of peroxidase action. For light emission, the luciferase of this system can be replaced by horseradish peroxidase, and the luciferin can be replaced by luminol.

The firefly system has been the subject of more intensive investigation than that of any other luminous organism studied thus far. The major points of interest regarding the purified system in vitro were treated at length by McElroy and Seliger. Among other noteworthy points was the evidence that the color of light emitted depends on the conformation state of the protein enzyme, firefly luciferase, which is alterable by various factors. The color of light is modified also by substituents in the luciferin substrate molecule. With reference to the flash emitted by the firefly photogenic organ, Buck discussed the problem with special reference to the basic

unit of light emission and the relation between the flash and the fine structure of the organ. Hagins, Hanson, and Buck noted that the steady glows of firefly organs and of luminous bacteria do not involve scintillations lasting less than 0.1 second.

The biochemical fractionation and final purification of essential reactants in luminescence systems is an approach of special interest, inasmuch as only four systems—those of the firefly, *Cypridina*, certain jellyfish (*Aequorea*), and probably luminous bacteria—have been fully purified thus far, and their components are all chemically different from each other. The following data touch on new advances along this line which were reported at the conference.

Kuwabara and Wassink described a purification procedure for the active substance of fungal luminescence (*Omphalia flavida*, now *Mycena citricolor*) which may be provisionally considered fungal luciferin, in a yield of 12 mg of crystalline product from 15 kg of wet mycelium. The absorption, fluorescence, and bioluminescence emission spectra of the substance were reported. Also noted was the ability of this substance to undergo chemiluminescence in absence of enzymes. Cormier and Totter reported a partially purified substance capable of bright phosphorescence in water-free acetone, obtained from the mycelium of the same species.

Isolation of the luciferin from the only known luminous animal completely indigenous to fresh water, namely the New Zealand limpet *Latia neritoides*, was reported by Shimomura, Johnson, and Haneda. This luciferin turned out to be unusual in consisting of an oil readily soluble in *n*-hexane. Its molecular weight by mass spectrometry is 236. Alcoholic solutions are non-fluorescent and colorless, with a single absorption maximum at 212 mμ.

Isolation of the longtime enigmatical system of the luminescent polychaete annelid worm *Chaetopterus* was reported by Shimomura and Johnson. It constituted the second example of a system comprised apparently of only one organic component, a bioluminescent protein, for which the general term "photoprotein" was suggested. The general terms luciferin and luciferase do not apply to such a system, at least in their usual meaning. The first example of a system of this nature was recently found in certain jellyfish (*Aequorea* and *Halistaura*). For luminescence in aqueous solution, the *Chaetopterus* photoprotein specifically requires ferrous iron, a peroxide, and oxygen, whereas the jellyfish photoprotein requires only calcium or strontium.

Partial purification and some properties of the luminescence system of a deep sea shrimp, *Hoplophorous*, was described by Johnson, Stachel, Shimomura, and Haneda. Shimomura, Johnson, and Haneda reported experiments with acetonized and partially ground photogenic organs of the "New Zealand glowworm" (*Arachnocampa luminosa*). The results suggest a biochemical relation, however remote, of this system to that of the firefly, inasmuch as ATP stimulated and pyrophosphate inhibited the light-emitting reaction. The same authors reported some observations on the luminescence system of the exceptionally large New Zealand earthworm (*Octochaetus multiporus*).

The other papers dealt primarily with morphological aspects of photogenic cells, tissues, and organs. Haneda described the luminous organ of the Australian pinecone fish *Cleidopus gloria-maris* and succeeded in obtaining pure cultures of the symbiotic luminous bacteria which provide the source of light in this organ. Through a critical study with the electron microscope of the photogenic organs of squids, Yô K. Okada demonstrated that rod-like contents, which had previously been interpreted as symbiotic bacteria by some authors, were definitely non-bacterial in nature. An extensive investigation, involving electron microscopy as well as conventional cytological and histological methods, provided the basis of an exposition by J.-M. Bassot on photogenic structures in general. Among the many points of interest was the evidence for a "morphological dualism," analogous or corresponding to the "biochemical dualism" of luminescence systems, in the sense that in virtually every example from simplest photocytes to the most complex photophores, there exists at least two different structural components or arrangements to which could be attributed the function of secreting or separating two different, active products, such as a luciferin and a luciferase. Of more than ordinary interest also was the discussion in this paper regarding the evolution of photophores.

The conference was jointly sponsored by the Japan Society for the Promotion of Science and the National Science Foundation. Proceedings of the conference will be published by Princeton University Press in a book entitled *Bioluminescence in Progress*; editors are F. H. Johnson and Y. Haneda.

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Forthcoming Events

September

21–23. **Molecular Motion** in Solids, Liquids, and Gases by Magnetic Resonance, mtg., Canterbury, England. (E. F. W. Seymour, British Radio Spectroscopy Group, School of Physics, Univ. of Warwick, Coventry England)

21–23. **Nuclear and Particle Physics**, conf., Univ. of Glasgow, Glasgow, Scotland. (Meetings Officer, Inst. of Physics and the Physical Soc., 47 Belgrave Sq., London S.W.1, England)

21–23. **Origin and Abundance-Distribution of the Elements**, symp., UNESCO headquarters, Paris, France. (W. D. Page, Div. of Earth Sciences, Natl. Acad. of Sciences 2101 Constitution Ave., Washington, D.C. 20418)

21–23. **Origin and Distribution of the Elements**, symp., Paris, France. (E. Ingerson, Dept. of Geology, Univ. of Texas, Austin 78712)

21–23. **Supermolecular Structure in Fibers**, 25th conf., Fiber Society, Boston, Mass. (L. Rebenfeld, Textile Research Inst., P.O. Box 625, Princeton, N.J.)

21–24. New Methods of **Stellar Dynamics**, colloquium, Besançon, France. (Assistant Secretary, Intern. Astronomical Union, Observatory of Nice, Le Mont-Gros, Nice, France)

21–29. International **Atomic Energy** Agency, 10th general conf., Vienna, Austria. (IAEA, Kärntnerring 11, Vienna 1)

22–24. American College of **Cardiology**, regional mtg. Univ. of Florida, Gainesville. (M. W. Wheat, Jr., Div. of Postgraduate Education, Univ. of Florida College of Medicine, Gainesville 32601)

22–24. **Muscle Circulation**, symp., Smolenice, Czechoslovakia. (O. Hudlická, Inst. of Physiology, Czechoslovak Acad. of Sciences, Budějovická 1083, Prague 4)

23–1. American Soc. of **Clinical Pathologists**, Chicago Ill. (Secretary, 445 North Lake Shore Dr., Chicago 11, Ill.)

24–26. **Phage Genetics and Physiology**, mtg., Naples, Italy. (Organizing Committee, Intern. Laboratory of Genetics and Biophysics, Naples)

25–28. **Gastrointestinal Radiation Injury**, symp., Richland, Wash. (M. F. Sullivan, Biology Dept., Battelle-Northwest, P.O. Box 999, Richland 99352)

25–29. **Water Pollution Control** Federation, 39th mtg., Kansas City, Mo. (R. E. Fuhrman, 4435 Wisconsin Ave., NW, Washington, D.C. 20016)