Bioluminescence

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PREFACE

The production of light without heat by living things has always appealed to the imagination and excited the interest of mankind. As a remarkable example of functional activity in animals and plants, bioluminescence itself not only presents many problems but has also become an important means of understanding vital processes in general. This is due in part to the development of highly accurate and rapid devices for recording weak lights and in part to a realization that luminescence intensity is a direct measure of the velocity of oxidative enzyme reactions intimately connected with the life of the cell. The light emitted by cells or cell extracts under various conditions can serve as a tool of great value for quantitative biophysical and biochemical investigation. This type of research has already yielded important results in the analysis of enzyme kinetics, drug action, temperature, and pressure effects.

Three types of luminous organisms have been extensively studied—the bacteria, the ostracod crustacean, Cypridina, and fire-flies—but they represent only a small proportion of the approximately forty additional groups which contain luminous species. Since many of the other little known luminous forms offer special advantages for chemical and physiological work, the author has collected the facts concerning them and reviewed the present knowledge of all groups of luminous organisms in this volume—a comprehensive monograph of the reference type. It is intended as a complete guide to knowledge on the subject. Biological aspects of light production have been included and the direction for future research suggested.

The compilation has not been an easy task. Fortunately, however, it is neither necessary nor desirable to trace the ideas regarding light emission by living organisms to the beginnings of scientific thought but only to mention the pioneers of the previous century. In this way due credit can be given to discoveries which are often overlooked or for which space is unavailable in the modern technical journal.

The plan of the book is simple. The various luminous groups have been treated in phylogenetic order as biological entities, with a short discussion of the luminous species and their habits, followed by a statement of the known facts concerning morphology, histology, physiology, bichemistry, and biophysics of light-producing cells or organs. Where the results of previous work are too extensive to warrant inclusion, references will be found in the Bibliography.

At one time it was hoped that lists of all known luminous animals in each group might be published, but problems of synonymy and space considerations have prevented carrying out the plan. In referring to luminous organisms, no attempt has been made to bring the nomenclature completely up to date. Whatever the latest scientific name of a particular species may be, it will be designated by the name used in the original publication dealing with its luminescence.

It is a pleasure to acknowledge the advice and help given me in discussion with my former students, especially Frank H. Johnson and Wm. D. McElroy, and my colleagues, Aurin M. Chase and John B. Buck, whose important researches on bioluminescence have done so much to advance the subject. Sincere thanks are due to many who have identified species or advised on systematic matters: Wm. W. Diehl, Edith K. Cash, D. P. Rogers, and Ruth Macrae on Fungi; the late C. A. Kofoid on Protozoa; Elizabeth Deichmann on Cnidaria and Ctenophora; R. S. Bassler on Bryozoa; J. P. Moore and Grace E. Pickford on Annelida; Waldo L. Schmitt, F. A. Chace, Jr., W. L. Tressler, C. R. Shoemaker, the late W. M. Tattersall, and the late C. B. Wilson on Crustacea; R. V. Chamberlin and H. F. Loomis on Myriapoda; E. A. Chapin, G. Vogt, P. J. Darlington, and the late H. S. Barber on Coleoptera; P. Bartsch on Mollusca; G. E. Pickford and W. J. Rees on Cephalopoda; the late H. L. Clark on Echinodermata; W. G. Van Name and N. J. Berrill on Tunicates; J. T. Nichols, A. E. Parr, and T. H. Watermann on Pisces; to those who have lent photographs or drawings, cited in connection with the figures, particularly to the Princeton University Press for permission to reproduce some of the figures of "Living Light"; to the librarians whose aid in finding the older references has been invaluable: Mrs. V. T. Phillips of the Academy of Natural Sciences, Philadelphia, Miss Hazel Gay of the American Museum of Natural History, New York, Mrs. Gertrude Hess of the American Philosophical Society library, Mrs. Deborah Harlow of the Marine Biological Laboratory library, Woods Hole, Mass., and Miss Genevieve Cobb of the Biology library of Princeton University; to my secretary, Mrs. T. M. Page, for valuable assistance in typing manuscript and checking references; and particularly to my wife, Dr. Ethel Browne Harvey, who has aided in translation of foreign languages, read the manuscript, and advised in many ways.

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INTRODUCTION

Perhaps the most striking biological fact regarding the emission of light by animals and plants is the great number of totally unrelated and diverse organisms which have developed this ability. Although the ratio of number of luminous species to the total number of known species is vanishingly small, the ratio of phyla or classes containing luminous animals to the total recognized phyla or classes is surprisingly large. Exact figures will depend on the classification used, since different zoologists recognize between 10 and 33 phyla. The official American list* of phyla, classes and orders of the animal kingdom represents one extreme. It contains 33 phyla and 80 classes. Among these 13 of the phyla (with two dubious) and 28 of the classes (with 3 dubious) contain luminous species.

The author has followed a classification** intermediate between the two extremes, containing 25 phyla, in 12 or 13 of which are to be found luminous species. This classification, with its 13 luminous phyla and some 28 luminous classes in italics, follows:

Protozoa
- *Plasmodroma*
  - *Flagellata* or *Mastigophora* (flagellates)
  - *Rhizopoda* or *Sarcodina* (rhizopods)
  - Sporozoa (sporozoans)
- Ciliophora
  - Ciliata (ciliates)
  - Suctoria (suctorians)

Mesozoa or Moruloidea
  - Rhombozoa or Dicyemida (Dicyema)
  - Orthonectida (Rhopalura)

Porifera (sponges)
  - *Calcarea* or *Calcispongiae* (calcareous sponges)
  - Hexactinellida (glass sponges)
  - Demospongiae (naked or horny sponges)

Cnidaria
  - *Hydrozoa* (hydroids)
  - *Scyphozoa* (medusae)
  - *Anthozoa* (corals, sea pens, etc.)

* Zoological Names," prepared by A. S. Pearse for Section F. A.A.A.S. Durham, N.C., 1949.

** From "Selected Invertebrate Types," edited by F. A. Brown, Jr., John Wiley Sons, New York, 1950.

Ctenophora (comb-jellies)
    Tentaculata
    Nuda (only Beroidae)
Platyhelminthes
    Turbellaria (flat worms)
    Trematoda (flukes)
    Cestoda (tape-worms)
Rhynchocoela or *Nemertinea* (nemerteans)
Acanthocephala (spiny-headed worms)
Aschelminthes
    Rotifera (rotifers)
    Gastrotricha (Chaetonotus)
    Kinorhyncha or Echinodera (Echinoderes)
    Priapulida (Priapulus)
    Nematoda (Nematodes)
    Nematomorpha or Gordiacea (hair-worms)
Entoprocta or Kamptozoa
? *Ectoprocta* or *Polyzoa* (bryozoans)
    ? *Gymnolaemata*
    Phylactolaemata
Phoronidea (Phoronis)
Annelida
    Polychaeta (marine worms)
    Archiannelida (archiannelids)
    Oligochaeta (earthworms)
    Hirudinea (leeches)
Echiuroidea
    Echiurida (Echiurus)
    Saccosomatida (Saccosoma)
Sipunculoidea (Sipunculus)
Mollusca
    Amphineura (Chaetoderma and Chiton)
    Scaphopoda (tooth shells)
    Pelecypoda or *Lamellibranchiata* (clams, etc.)
    Gastropoda (snails)
    Cephalopoda (squid)
Brachiopoda (lamp shells)
    Inarticulata
    Articulata
Onychophora (Peripatus)
Arthropoda
  Chelicerata
    Merostomata or Xiphosura. (Limulus)
    Pycnogonida or *Pantopoda* (sea spiders)
    Arachnida (spiders, scorpions, etc.)
  Mandibulata
    Eucrustacea (crustaceans)
    Pauropoda (Pauropus)
    Diplopoda (millipedes)
    Symphyla (Scolopendrella)

Chilopoda (centipedes)
Insecta (insects)
Linguatulida (Linguatula)
Tardigrada (water bears)
? *Chaetognatha* (Sagitta)
Echinodermata
Eleutherozoa
? *Asteroidea* (star-fish)
Ophiuroidea (brittle stars)
Echinoidea (sea urchins)
Holothuroidea (holothurians)
Pelmatozoa
Crinoidea (crinoids)
Enteropneusta or *Hemichorda*
Balanoglossida (Balanoglossus)
Cephalodiscida or Pterobranchia (Cephalodiscus)
Chordata
Tunicata or *Urochorda* (tunicates)
Larvacea (Appendicularia)
Ascidiacea (ascidians)
Thaliacea (Salpa, Pyrosoma)
Cephalochorda or Leptocardia (Amphioxus)
Vertebrata or *Craniata* (vertebrates)
Cyclostomata (cyclostomes)
Pisces (fish)
Amphibia (amphibia)
Reptilia (reptiles)
Aves (birds)
Mammalia (mammals)

In later chapters the author has, unless otherwise specified, used the subdivision into orders, families, and genera given in the "Handbuch der Zoologie" edited by W. Kükenthal and T. Krumbach.

In the plant kingdom, there has been more agreement on large divisions. Most botanists recognize 4 subkingdoms and about 9 divisions. If the dinoflagellates are considered animals, the only luminous plants* are to be found among the bacteria and the higher fungi, which means that a definitely smaller per cent of plant groups have developed the ability to emit light. The distribution in the plant kingdom can be seen from the following simple scheme in which the groups containing luminous species are italicized.

Thallophyta
Algae
Cyanophyceae (Blue-green Algae)
Chlorophyceae (Green Algae)

* Recently Strehler and Arnold (1951) have described chemiluminescent light emission of very low intensity accompanying photosynthetic activity.

Phaeophyceae (Brown Algae)
Rhodophyceae (Red Algae)
Fungi
Myxomycetes (Slime moulds)
Schizomycetes (Bacteria)
Phycomycetes (moulds)
? *Ascomycetes* (Sac fungi, yeasts, some moulds)
Basidiomycetes (Smuts, rusts, mushrooms)
Bryophyta
Hepaticae (Liverworts)
Musci (Mosses)
Pteridophyta
Equisetineae (Horsetails)
Lycopodineae (Club Mosses)
Filicineae (Ferns)
Spermatophyta
Gymnospermae (Cycads, Ginkgo, Conifers)
Angiospermae (Mono- and Dicotyledonous flowering plants)

It is apparent from the previous classifications that no clear develop ment of luminosity along evolutionary lines is to be detected but rather a cropping up of luminescence here and there, as if a handful of damp sand has been cast over the names of various groups written on a blackboard, with luminous species appearing wherever a mass of sand struck. The Ctenophora have received the most sand. It is probable that all members of this phylum are luminous. The Cnidaria also contain many luminous species scattered among certain of the orders.

At the other extreme are very large groups in which only a few luminous animals are known, as in the gastropod and lamellibranch molluscs. It is an extraordinary fact that one species in a genus may be luminous and another closely allied species may contain no trace of luminosity. Only among animals with complicated luminous organs or photophores, such as shrimp, squid, and fish, does there appear to be a definite series of gradations with increasing complexity, that might be regarded as an evolutionary line. Elsewhere the ability to emit light must have arisen independently in widely scattered groups.

Another striking peculiarity of luminescence distribution is the almost complete absence of luminous species in fresh water. The most striking instance of this rule is to be found among dinoflagellates in which only the salt water species can emit light. The only true exception known at the present time is the fresh water limpet, Latia, of New Zealand. Possibly aquatic fire-fly larvae of inland pools and streams and fresh water luminous bacteria, which sometimes grow within living fresh water shrimp can also be called exceptions.

Although luminous species are abundant in the depths of the ocean, they do not occur in the depths of inland lakes or in the fresh water of caves. Parasitic luminous species (except bacteria) are also unknown, and among terrestrial animals luminosity cannot be connected with any peculiarity of habitat or relationship, except that luminous forms are almost universally nocturnal.

There is no doubt whatever but that bioluminescence is a form of chemiluminescence in which definite chemical substances emit the light during a chemical reaction. The process can be completely imitated by organic compounds of known composition in the laboratory. Luciferin and luciferase are general names used for these compounds manufactured by luminous animals, but it is probable that the luciferin or luciferase from a species in one group may be quite different chemically from that in another.

The light emission of living things may be intracellular or appear only after the luminous materials have been secreted to the exterior (extracellular luminescence). The fine structure of the luminous cells or luminous organs may be very different in different groups. In some cases the luminous organ may be so complicated by accessory structures, like lenses, reflectors, or color screens, that the whole makes up a veritable lantern. Detailed information on all aspects of the light-emitting process in various groups of animals and plants will be found in the following pages.

CONTENTS

Chapter I

Bacteria

Saprophytic Luminous Species

Whenever dead fish, flesh of animals of all kinds including man, eggs, sausages, and various dead invertebrates become luminous, it is practically certain that the light results from the growth of luminous bacteria. If the luminous material is dead vegetable matter, wood, roots, beets, potatoes,[1] fruit,[2] etc., the light is usually due to luminous fungi. Phosphorescence of flesh was known to Aristotle and has aroused interest and curiosity among the learned from the sixteenth century on. The history of attempts to explain the origin of the light makes fascinating reading, but space limitations allow only a few important discoveries to be recorded here.

Possibly the first hint that phosphorescence of dead fish and flesh might be due to living things is due to Baker in 1742. In his book, The Microscope Made Easy, he suggested that the light might come from "animacules," just as he had found the light of the sea to be due to tiny "insects."

Many of the fundamental discoveries regarding the properties of bacterial light were made by men who did not know that living matter was involved. Boyle, using his air pump in 1668, noted the dependence of the light of both wood and fish on the air and hence on oxygen, although oxygen as an entity was not known at the time. Martin, in 1761, discovered the necessity of salt for marine luminous bacteria although he thought he was studying phosphorescence of the sea. Canton in 1769, during his study of luminous fish, was the first to observe the reversible extinction of the light of bacteria by rise in temperature, although he little realized the importance that this effect was

[1] Cooked potatoes sometimes serve as culture media for luminous bacteria (see Molisch, 1904, 12) and luminous cheese may be due to luminous fungi, but the actual cause has not been determined.

[2] Göbel (1824) reported luminescence of bubbles of CO_2 accompanying the fermentation of raspberry juice, a phenomenon possibly due to luminous bacteria.

to assume in modern studies on the kinetics of light production. Canton wrote, "putting a very small piece of a luminous fish into a thin glass ball, I fouud that water of the heat of 118 degrees would destroy its luminousness in less than half a minute; which on taking it out of the water, it would begin to recover in about ten seconds, but was never so bright as before." Other names associated with early observation of bacterial luminescence are Cardanus, Fabricius, Borelli, Bartolin, Beale, and Rede. The prize essays of Dartous de Mairan in 1717 and Cohausen also in 1717, the book of Priestley in 1772, and later prize essays of Bernoulli (1803), Link (1808), Heinrich (1808, 1820), and Dessaignes (1809) all speculate on what was then known concerning the light of fish and flesh.

In the late eighteenth and early nineteenth centuries there were further observations (Delius in 1784; Hulme, 1800, 01; Deslongchamps, 1838) on dead fish, flesh, and a number of experiments on wood to explain the origin of the light. Most of them considered it to be connected with decay and decomposition, more specifically to combustion of some organic compound similar to phosphine. It was Heller in 1853 who definitely named an organism, Sarcina lutea, as the cause of the light of meat. Heller showed that new flesh could be inoculated with the luminous material[3] and Pflüger in 1875 demonstrated that the bacteria from fish could be filtered off and would grow on culture media. He did not give them a name, but the omission was rectified by Cohn (1878) who proposed the name, Micrococcus phosphoreus.

It is interesting to note that a number of workers (Phipson, 1860, 70; Mulder, 1860; Hankel, 1862; Horne, 1869) between 1853 and 1875 were still unaware of the living nature of the luminescence of dead fish and flesh. One of these was Panceri (1871), who, despite his great contributions to knowledge of many luminous invertebrates, regarded the light from a dead fish, Trachopteris iris, which he studied, as due to oxidation of fat.

Subsequent to Pflüger's paper, publications were largely concerned with naming the bacteria. Nuesh (1877, 79) described Bacterium lucens and B. termo, while Bancel and Husson (1879) found two varieties of bacteria growing on lobsters which they thought produced "carburetted and phosphoretted hydrogen" responsible for the light. Lassar (1880) confirmed Pflüger's work and spoke for micrococci which he considered responsible for the luminescence of dead invertebrates.

[3] Cooper and Cooper (1838) found that luminous material on human cadavers would cause other non-luminous cadavers to become luminous when rubbed over them.

An early experimental investigation was that of Ludwig (1882, 1884) who described Micrococcus Pflügeri and many of its characteristics, including its spectrum. The light appeared pale green, extending from the Fraunhofer line b into the violet. Luminous bacteria had become of great interest to the bacteriological and biological world. Ludwig (1887, 92) continued the study of photogenic bacteria and was followed by a number of workers, Dubois (1885), Fischer (1886, 87, 88), Hermes and Forster (1887), Tilanus (1888–90), and a review by Duclaux (1887).

The most comprehensive study of species was by Fischer, a ship's medical officer, and an early worker in the whole field of marine bacteriology. He discovered Bacterium phosphorescens from the West Indies and another species from the Baltic Sea and later (1894) isolated nine new species while on the Humboldt-Plankton expedition, and studied not only cultural characteristics but the general properties of these forms. He was followed by Forster (1887, 92) in Amsterdam, who again observed the spectrum and also noted a slow growth even at the freezing point. Lehmann (1889) and Tollhausen (1889) continued the work on Bacterium phosphorescens of Fischer. In the meantime Katz (1887, 91) described in detail in a long paper the characteristics of Bacillus cyaneo-phosphorescens, B. smaragdino-phosphorescens, B. argenteo-phosphorescens I, II, and III and B. argenteo-phosphorescens liquefaciens, all from Australia. It is apparent that luminous bacteria are of worldwide distribution.

Dubois (1888–93, 1919) studied Photobacterium sarcophilum, Bacterium pholas and B. pelagia, Beijerinck (1889–91) Photobacterium luminosum, Ph. indicum, Ph. fischeri, now called Achromobacter fischeri, and Ph. phosphorescens = Ph. pflügeri. Giard (1889, 90) and Giard and Billet (1889, 90) discovered a Diplobacterium (later called Bacterium giardi) infecting sand fleas, and Eijkmann (1892) isolated Photobacterium javanense from fish in Java. One of the most interesting and important discoveries was made by Kutscher (1893, 95) who found a luminous Vibrio from the river Elbe, similar to the cholera vibrio, thus establishing the existence of fresh water species. Weleminsky (1895) actually isolated luminous Vibrios from patients with Asiatic cholera. The strain was not luminous at first but became luminous after passage through pigeon's blood.

Review or general papers were published by Dubois (1889), Hericourt (1890), Cotton (1891), Clautrian (1896), Migula (1897), Barnard (1899, 1902), and A. Fischer (1900). Barnard's paper is particularly complete, listing 26 different species described up to 1899, giving his own experience with the properties of 12 of them and describing a new organism, Photobacterium liquifaciens Plymouthii. Barnard expressed the belief that many of the bacteria previously described were the same or very similar organisms and emphasized the fact that luminescent bacteria tend to undergo involution and exhibit pleomorphism "presenting at one time a rod shape and at another time assuming the form of a spirillum, whilst mixed forms are not infrequent."

At the beginning of the nineteenth century, Molisch (1902–1905, 12, 25) started his experiments on luminous bacteria from various sources, hen's eggs,[4] sausages, the water of Trieste harbor—which are summarized in the two editions (1904, 12) of his book, Leuchtende Pflanzen. He listed 26 well-described species of luminous bacteria in the 1904 edition and 30 in the 1912 edition. About the same time new bacteria were discovered by Tarchanoff (1901), Nadson (1903, 08), Foa and Chiapella (1903), Immaura (1904), Gorham (1904), Ugloff (1908), and particularly Reinalt (1906), who made a careful study of the cultural differences of many different types. Issatschenko (1911) found Bacterium hippanis on fresh water fish from the southern Bug River and Beijerinck (1912–15) continued his work on various forms, paying particular attention to the development of new strains. It is apparent that a very extensive variety of luminous bacteria was known early in the twentieth century.

It is not possible to list all the new species of luminous bacteria described in later years. Many of these bacteria have been parasitic on or symbiotic with various animals, making the host luminous while living. These will be considered in special sections. A few bacteria have been obtained from the flesh of dead animals or from fresh or salt water. Among them may be mentioned Coccobacillus sp. of Fejgin (1926), Pseudomonas luminescens, P. photogena, and P. phosphorescens of Kishitani (1928, 33), forms from the Black Sea and Sea of Azof (Egcrowa, 1929), Bacterium photodoticum of Vouk, Skoric, and Klas (1931), Vibrio phosphorescens of Maslennikowa (1927), and Stutzer (1929), V. albensis of Sonnenschein (1931, 32), new species described by Fuhrmann (1932), and Achromobacter harveyi of Johnson and Shunk (1936). Yasaki and Nomura (1947) described luminous forms from the digestive organs of fish, and Baylor[5] has isolated a number of species with unusual properties from the alimentary tract of deep sea fish obtained at Bermuda. Many observa-

[4] Molisch (1905) has made a special study of the reported cases of bird and reptile egg luminescence and particularly of cooked eggs which are the result of luminous bacterial infection. Haga (1942) in Japanese, has found the penetration of luminous bacteria through the shell but not the membrane of the hen's egg.

[5] Private communication.