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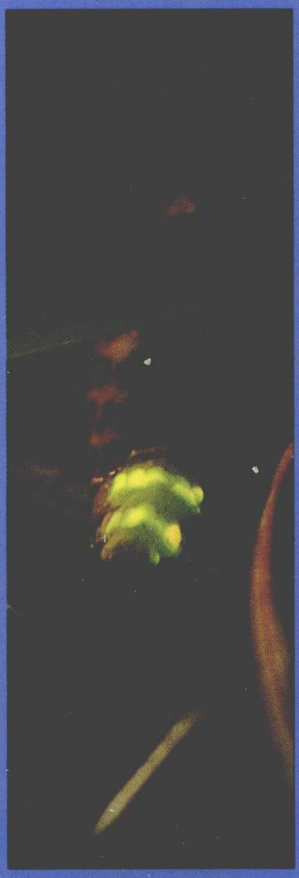
A. K. Campbell

Chemiluminescence

Principles and Applications in Biology and Medicine



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ELLIS HORWOOD
international publishers in science and technology

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Preface

> These *fluorescent* animals you collect on the beach at night are rather rare aren't they? Is this something you do just for fun? How on earth are they connected to your investigations into rheumatoid arthritis and multiple sclerosis?

This book is about chemiluminescence, chemical reactions that produce light. Those of us who enthuse over such reactions have, I am sure, faced many times questions such as these from family and friends. They highlight several areas of confusion and ignorance in both the layman and non-expert scientist. Many people are confused about the difference between fluorescence and chemiluminescence. These are related phenomena, but quite distinct. Switch the illumination of a home aquarium off and the beautiful blue neon fish cease to glow. Yet there are other fish in the oceans which can generate their own light. To find them, you have to dive around a coral reef at night or send a net several hundreds of metres below the ocean surface. Luminous animals may be relatively uncommon on land but in the sea they are abundant. You will find a few, such as dinoflagellates, bacteria on decaying flesh, worms and hydroids, on most beaches in the world. Over half of the earth's surface is covered by ocean more than 1000 metres deep. Here the only light is living light. Why otherwise would the animals here have retained their eyes fully intact through evolution? Bioluminescence, as it is known, thus plays a major role in the ecology of the oceans.

Recently, chemi- and bio-luminescence have had an impact on biochemical analysis, on cell biology, and on medicine. The light-emitting proteins from luminous jelly fish enable us to measure free calcium in living cells. This has led not only to fundamental discoveries about the role of intracellular calcium in controlling the behaviour of cells in healthy tissues but also about its role in cell pathology. It has lead us to propose a role for intracellular calcium in cell injury induced by cells and components of the immune system, relevant to demyelination in multiple sclerosis. The firefly system is now a standard ultrasensitive method for measuring ATP, an indicator of cell viability and biomass, since all living cells must have high ATP, but dead cells do not. Chemi- and bio-luminescent compounds have also had a recent impact on biotechnology, as replacements for radio-active labels such as ^{125}I and ^{32}P.

The markets involve thousands of millions of dollars per year on a world-wide basis! In spite of this fundamental importance and exciting application in biomedicine, most standard chemistry, biology, physiology, and biochemistry textbooks either do not mention chemiluminescence at all or deal with it scantily.

Possibly one of the earliest descriptions of bioluminescence is to be found in *Shih Ching* or *Book of odes*, one of the Thirteen Classics of China dating from around 1500–1000 BC. Luminous animals and fungi were described by Greek and Roman authors, such as Aristotle and Pliny. However, serious scientific investigation did not begin until the 16th century. The first book devoted entirely to luminescence was written by Conrad Gesner in 1555. Translated, the title reads: *On the rare and marvellous plants that are called lunar either because they shine at night or for other reasons; and also on other things that shine in darkness.*

Since then there have been just a few monographs by a single author devoted entirely to chemiluminescence, the most notable being by Horace Raphael Dubois in 1914 (*La vie et la lumière*, 338 pp, Paris), and the magnificent collection by E. Newton Harvey, including his scholarly account of some 800 plus pages on the history of luminescence. More recently, there have been several multi-author volumes, and conference proceedings, dealing with both fundamental and applied aspects of chemi- and bio-luminescence. So, even if there has not been a single-author text on this subject for more than 30 years, how can I justify this one? Surely one person, however enthusiastic, cannot readily do justice, and cover in depth, a field which stretches from the biology of the deep oceans, through the biochemistry, chemistry, and physics of reactions producing an excited state and their application to human good, to the nature of light itself?

It is my belief that science, both its practice and presentation, is not only about in-depth experimentation and consensus, but also, like the arts, about individual expression through 'thought, word, and deed'. The ever-increasing specialisation in science and fragmentation of disciplines may help one to find a single focus, but, unless one is careful, it may give us only a rather narrow perspective on Nature. The first true scientists were natural philosophers, not only capable of tussling with a single problem, but also having the wisdom to step back and view its wider perspective. They recognised the importance of getting the philosophy right if one was to appreciate Nature in all its aspects.

The study of chemiluminescence provides us with an ideal opportunity to return to, and yet develop further, natural science as a whole. It also provides illuminating insights into the historical development of science. Everyone at school learns Boyle's Law. Yet how many know that Boyle was the first to demonstrate the need for oxygen, some 100 years before its discovery, in bioluminescence? Who has heard of Horace Raphael Dubois? Not only did his experiments form the foundation for the investigation of the biochemistry, and chemistry, of bioluminescence over the past 100 years, but also he was one of the pioneers of modern biophysics.

The aim, therefore, of this book is to provide a wide, but balanced, perspective on chemical reactions which produce light: their discovery, where they can be found, and what is so special about a chemiluminescent reaction (Chapter 1), how to study and measure it (Chapter 2), its biology (Chapter 3), and its use as a laboratory tool in research and biotechnology (Chapters 4–9). Any technique in science is only as powerful as the questions asked by the user. I have, therefore, attempted to highlight

the unique aspects of chemiluminescence in analysis, and to pinpoint where real discoveries have arisen from its use in biology and medicine. The last chapter looks to the future, and summarises particularly my own enthusiasm for studying chemiluminescence. I have deliberately repeated a few points in more than one chapter, so that each may be read independently.

The book is aimed at researchers and undergraduates in the biological disciplines including medicine, and in chemistry. In view of the exciting prospects for commercial applications of chemiluminescence I have tried to highlight these in such a way that the text will also be of interest to industrialists. The style I have adopted is first to try to ask simple questions fundamental to an understanding of the phenomenon, and then to delve more deeply into its chemistry, physics, and biology. I have tried to explain how things work, including the apparatus. Walk into any laboratory and you are likely to find at least one piece of equipment whose analytical basis involves the measurement of light. Yet how many students know how a photomultiplier, or other types of light detector, work? Scientists must understand their tools. This is essential if we are to realise the strengths and weaknesses of our current experimental approach to a problem, and highlights the need for inventing new devices and new analytical procedures.

My first introduction to chemiluminescence analysis was some twenty years ago whilst studying with Dr (now Professor) Nick Hales, for my PhD, in the Department of Biochemistry, University of Cambridge. I needed to measure tiny amounts of ATP, and had not a great deal of success using a fluorescence assay. Another member of the group, Paul Luzio, now a close friend and collaborator, drew my attention to a paper which showed how the firefly luciferin–luciferase reaction control be used to measure ATP. I was surprised, but delighted, to find that a firefly extract was available commercially. My first assay was a revelation. It worked first time! Using a Heath Robinson scintillation counter I immediately succeeded in establishing an ATP assay some two to three orders of magnitude more sensitive than I had achieved previously. I owe a great debt to many other people for helping me with my research over the past 20 years. First I should like to thank all who have been members of my research group or who have interacted closely with it. Some are still with me, I am pleased to say: Trevor Baines, Peter Evans, Bob Dormer, Maurice Hallett, Steve Simpson, Richard Daw, Chris Davies, Ashok Patel, Ian Weeks, Alun Davies, Stephanie Matthews, Mary Holt, Steve Edwards, Paul Morgan, Ann Roberts, Jan Knight, Shirley Barrow, Ashvin Patel, David Jenner, Graciela Sala-Newby, and Alan Houston, Eryl Davies, Thomas Müller, and Miguel Lucas. I thank Malcolm E. T. Ryall for all his hard work constructing our chemiluminometers, and more recently, Peter Mason. I have been lucky enough to collaborate with many interesting people. I thank the Director, Professor Sir Eric J. Denton, FRS, and all the staff and my friends at the Marine Biological Laboratory, Plymouth, where I have spent so many happy and stimulating hours. Particular thanks go to Skipper Chris Knott and the crew of the *Gammarus* for their help in collecting in my first luminous animals, *Obelia geniculata*, and Commander Bax of Plymouth Ocean Projects and the UWIST sub-aqua club for providing divers to collect them. I also thank particularly Peter J. Herring, whose invitation to join cruises on RRS *Discovery* have enabled me to discover the wonders of living light. I also thank Professor Frank McCapra and his group for their much appreciated collaboration;

Chris Ashley for many enjoyable hours in his laboratory, Nick Hales and those originally in his group, particularly Ken Siddle, Paul Luzio, and Gerry Brenchley, and David Yates and Professor H. Gutfreund, FRS in Bristol, Jean-Marie Bassot for a happy trip to Station Biologique Roscoff, and Alan Walton and George Reynolds and Geoff Newman. I thank Nick Hales for much support, particularly in my early years, the late Leslie Cooper, FRS, for his wisdom, encouragement, and introduction to the Marine Biological Association, Plymouth, and also to his wife Daloni for their wonderful hospitality over the years. I am also very grateful for the long-standing support of the members of the Department of Medical Biochemistry, University of Wales College of Medicine (formerly Welsh National School of Medicine), particularly Professor George Elder for all his support, J. Stuart Woodhead, Bob Dormer, Noor Kalshekar, Paul Morgan, and Ian Weeks, and the secretaries, Chris Hullin, Dot Thomas, Dilys Marks, and Jeannie Burt. Goodness knows how they have managed to read my writing or cope with me over the years; their support has been much appreciated. I am also grateful to Andrew Newby, Alan Mcgregor, Ralph Marshall, Tony Jackson, and particularly Alastair Compston for their interest and collaboration, and the department of Medical Illustration and the Animal House for their invaluable support.

I owe many people thanks for material in this book, but particularly Peter Herring for some of the photographs of luminous animals, Woody (J. W.) Hastings for his most generous gift of one of my treasured possessions, a copy of Harvey's *A history of luminescence* (1957), Professor G. Pérès for the photograph and review of Dubois, and Professor George Reynolds for the photograph of E. Newton Harvey.

My research has been generously supported by the Medical Research Council, the Science and Engineering Research Council, the Arthritis and Rheumatism Council, the Multiple Sclerosis Society, the Department of Health and Social Security, The Welsh Office, the Browne and Maurice Hill Bequest Fund of The Royal Society and CLEAR Ltd. Without their financial support I would not have had the opportunity to discover and exploit living light.

I am extremely grateful to Ellis Horwood Limited, particularly Sue Horwood, for inviting me to write this book. I am a pencil and paper writer, so very special thanks go to Rosemary Harris for typing the manuscript so excellently and accurately, in spite of its apparent illegibility.

Finally I thank my family for all their support: my wife Stephanie, my sons David and Neil, and my daughter Georgina, whom I treasure greatly. I thank Stephanie particularly for her marvellous support. Families of scientists have a lot to put up with. Mine have chased me across the beach or up a mountain at night, as I excitedly search for a new luminous animal. They have put up with me escaping to our Anglesey retreat, where I find endless inspiration. I also thank Sue Campbell.

My mother and father first encouraged me to pursue my embryonic interest in Nature. Thank you for everything and for many valuable discussions since with my sister and collaborator, Caroline Sewry.

Go, view that House, amid the garden's bound,
Where tattered volumes strew the learned ground,
Where Novels, — Sermons in confusion lie,
Law, ethics, physics, school-divinity;
Yet did each author, with a parent's joy,

Survey the growing beauties of his boy,
Upon his new-born babe did fondly look,
And deem Eternity should claim his book,
Taste ever shifts, in half a score of years
A changeful public may alarm thy fears;
Who now reads Cowley? — The sad doom await,
Since such *as these are now* may be thy fate.

Thus wrote Gilbert White to his friends and relatives in 1788 whilst nervously awaiting the publication of his life's work *The natural history and antiquities of Selbourne*. Few of us can expect our contributions to stand the test of time as White's has done. Yet we all go through similar anguish and self-doubt. What then is the real purpose of the present book? As with Gilbert White, it is part of my own personal quest for a better understanding of the natural world. It is an attempt to marry the aesthetic appeal of a truly remarkable phenomenon, with the intellectual challenge of learning more about it and harnessing it to human good. Hopefully others will find pleasure as well as stimulation in some of its pages.

Anthony K. Campbell
Cartrefle
Ynys Môn

October 1987