The Production of Light by Living Organisms^{*}

The Chemistry of Biophotogenesis

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THE term "Biophotogenesis," in its complete sense of the production of light by living organisms, covers a group of phenomena accompanying the vital processes in a wide range of animal and vegetable forms. The fireflies (*Lampyridæ*) are to us the commonest and most brilliant of these biophotogenic forms.

During the summer of 1909, the writer was associated with Professor Joseph H. Kastle, of the University of Virginia (then Chief of the Division of Chemistry of the Hygienic Laboratory), in a study of the effect of various chemical reagents on the luminous tissue of the firefly common in this locality (*Photinus pyralis* Linn.), and the results of our work are contained in a paper entitled "Some Observations on the Production of Light by the Firefly," in the *American Journal of Physiology*, Vol. 27, pp. 122-151. At the time this work was started, Professor Kastle was engaged in the preparation of his monograph on "The Oxidases and Other Oxygen-Catalysts Concerned in Biological Oxidations" (Bulletin No. 59, Hygienic Laboratory), and a latent interest in this subject was awakened by the recurrence of references to the claim of Dubois that the photogenic process in organisms involves the action of an oxidizing ferment or oxidase, to which this investigator had given the name "Luciferase." Professor Kastle's prior observations on this point had led him to believe that no oxidase was present, but that peroxidase and catalase were present; he found that aqueous extracts of the luminous tissue of the common firefly failed to turn tincture of gualacum blue, except in the presence of hydrogen peroxide, and the bluing in the presence of the latter was accompanied by the rapid disengagement of oxygen.

This insect, *Photinus pyralis* Linnæus, is the one which is so very common in our parks and lawns during the summer. It is a species of the family *Lampyridæ* (fireflies, lightning-bugs), of the genus *Coleoptera* (beetles). The luminous apparatus of the male insect—the more commonly seen of the sexes—occupies the entire ventral surfaces of the two abdominal segments next to the last, and a portion of the preceding segment. That of the female is a small rectangular area on the third abdominal segment from the last; both sexes have also two very small points of luminous tissue on the last abdominal segment.

The color of the light of this insect is generally stated to be yellow or yellowish-green; some of the other local species exhibit a light of a slightly different tone. The light of the *pyralis* has recently been made the subject of a very interesting spectrophotographic study by Drs. Ives and Coblentz, at the Bureau of Standards (*Bulletin of the Bureau of Standards*, Vol. 6, pp. 321-336). These observers found the light of this insect to resemble very closely the light of the Cuban cucuyo (*Pyrophorus noctilucus* Linn.), as studied and described by the late Professor S. P. Langley and Professor F. W. Very, nearly twenty years ago. ("The Cheapest Form of Light," Miscellaneous Collections, Smithsonian Institution, 1901; reprint.) Briefly, the spectrum of the light of *Photinus pyralis* consists of a "structureless, unsymmetrical band" in the red, yellow and lower blue portions of the visible spectrum, with a maximum at that portion having the greatest illuminating effect with the minimum of actinic and thermal effects. It gives no hint of continuation in the infra-red or ultra-violet portions of spectrum. It seems probable that the light of most luminous insects is essentially similar to that of the *pyralis*; two other species of *Lampyridæ* that I have examined have lights showing spectra of even more limited range than that of the *pyralis*, and in both of these species the color of the light to the eye is more greenish than that of the commoner insect. However, Mr. H. S. Barber, of the U. S. National Museum (Proceedings of the Washington Entomological Society, Vol. 9, pp. 41-43) and other observers have reported that various species of tropical *Phengodini* (a group of beetles probably closely allied to the *Lampyridæ*) give a red light from a photogenic organ in the head; no spectroscopic studies of this light have been made. Max Trautz ("Studien über Chemilumineszenz," *Zeitschrift für physikalische Chemie*, Vol. 53, pp. 1-111) states that the spectrum of the light produced by the oxidation of pyrogallol by means of thirty per cent. hydrogen peroxide in the presence of formaldehyde, extends from the red to the lower blue, and there-

fore covers very nearly the same spectral area as that of the light of the firefly. The spectrum of the light of glowing phosphorous also appears to give a spectrum lying within this same area, though of less extent than that of the firefly. Although a little ahead of the subject, I will state here that the light produced by moistening with 3 per cent. hydrogen peroxide solution the luminous tissue of the firefly which has been desiccated over sulphuric acid in an atmosphere of hydrogen, gives a spectrum of very limited range, extending from the orange to the yellow-green. The spectrum of the light produced by the photogenic bacteria is also of less range than that of the *Lampyridæ*.

It need scarcely be said that the light of the firefly affects the photographic plate; obviously spectrophotographic studies could not otherwise have been made upon it. Dubois has taken photographs by means of the light of the photogenic bacteria and of the cucuyo. In 1896 Muraoka (*Annalen der Chemie und Physik*, Vol. 295, pp. 773-781) announced that he had proved the penetration of metal films by means of the light of the firefly, in a manner similar to that of the X-rays. This was very puzzling to physicists, in view of the known limited range of the light, and the fact that it gave no other evidence of containing ultra-violet or penetrating rays. I have experimented with the local insect (*Photinus pyralis*), and have failed to find the least evidence of penetration of thin sheet copper, aluminium foil, or the ordinary black paper with which X-ray plates are wrapped. Some explanation of Muraoka's rather extraordinary results has been offered, based upon some more or less vague bacterial or vapor influence, but when we consider that his results were published only a little while after the discovery of the X-ray, it seems possible that Muraoka was just a little over-enthusiastic.

Professor Kastle and the writer tried the effect of a large number of chemical substances upon the live insect, the freshly detached luminous organ, and the luminous tissue which had been dried in hydrogen, and for these in detail the reader is referred to the paper in the *American Journal of Physiology*, ante. A great deal of work had already been done along similar lines, some of it dating back to the times of Reaumur and Spallanzani; Ehrenberg, Milne-Edwards and Pfüger have in turn reviewed this literature quite thoroughly.

Some of our results, however, seem worthy of special attention. Taking first the live insect: Injections of solutions of the metallic nitrates, of strychnin and of adrenalin, caused the emission of light. Immersion of the insect in methyl and ethyl alcohols, in ether and in chloroform, resulted in the production of light. Immersion in pure oxygen appeared to stimulate the photogenic function somewhat, but not as much as might have been expected. Immersion in nitrous oxide caused a considerable increase in the intensity of the light. In the cases of injection and immersion in liquids, the reagents kill the insect, but not until they have caused light-emission. Nitrous oxide narcotizes the insect, but in the air it recovers again. Hydrocyanic acid and cyanogen kill the insect, of course, but not until they have caused the emission of light. The luminous organ of one of the local species of *Lampyridæ* has been observed to glow in the mixture of air and prussic acid in the cyanide killing-bottle for over an hour, long after the actual death of the insect. Ammonia-water causes the evolution of light, either by injection or immersion; Watasé is authority for the statement that if a tissue suspected of being luminous refuses to give light with any other stimulus, it will, if a true photogenic tissue, glow on moistening with dilute ammonia-water. The injection of 3 per cent. hydrogen peroxide solution also caused the evolution of light.

With the freshly detached luminous segments, the most notable results were obtained with the vapors of methyl and ethyl alcohols, carbon tetrachloride and bisulphide, and mononitrobenzene, acting in the presence of air. All of these reagents caused light-emission and the light given out was not the continuous faint glow frequently the result of weak chemical stimuli, but was accompanied by a series of distinct flashes or pulsations of light, similar to the normal flashes of the insect. With the detached organ, the effect of powerful poisons was in almost every instance to produce the evolution of light, sometimes faint and of short duration, but definite.

As examples of poisons acting thus may be cited hydrofluoric acid, iodine cyanide and bromine.

The interesting fact that the photogenic tissue of luminous life-forms preserves after desiccation the power to evolve light on the application of water in the presence of air or oxygen, has long been known, and it at once suggests other known instances of the preservation of biologic activity by drying, as exemplified by the yeasts and ferments. By drying the photogenic tissue of *Photinus pyralis* over sulphuric acid in hydrogen or a hydrogen-vacuum, we have prepared dry material which has retained its photogenic activity apparently without loss, when kept in sealed tubes, for over thirteen months; indeed, there seems to be no good reason why, under these circumstances, it should deteriorate. In its conduct toward various chemical substances, the dried tissue, after moistening, does not differ essentially from the live insect or the freshly detached luminous organ. It glows on moistening in the air, somewhat brighter on moistening in oxygen, and but dimly or not at all when moistened in nitrogen, hydrogen and carbon dioxide. Moistened with 3 per cent. hydrogen peroxide instead of water, the dried tissue produces a much brighter light than with water alone, accompanied by the decomposition of the peroxide. The spectrum of the light thus produced has already been referred to.

Thus far, one substance alone has conducted itself as a positive inhibitor of the photogenic function. This is sulphur dioxide. Carradori observed this fact with the *Luciola italica* over one hundred years ago, Dubois has made a similar observation with regard to the cucuyo. The live insect, the freshly detached luminous organ, and the dried tissue, placed in this gas, all fail to glow, or glow but weakly and momentarily and are dead to all other stimuli when removed from it. As a rule even those substances which tend to poison the luminous tissue, caused the evolution of a dim light at first, but not so with sulphur dioxide in the majority of cases in which we used it.

Mechanical stimuli, such as friction and percussion, and physical stimuli, such as electricity, heat and light, also cause the production of light by the luminous organs of the firefly, whether attached to the living insect or detached. The effects of various temperatures and of electric discharges of various strengths have been extensively studied by other observers. Transferring the detached luminous organs from one gas to another, even though one or both be chemically neutral, may cause light-production, apparently due to some osmotic effect. Currents of air and other gases exert an effect on these detached organs, which Professor Kastle has compared to the effect of air-currents on the strychninized frog. It is obvious from these facts that the luminous tissue is one of great irritability.

Our knowledge of the chemical processes involved in biophotogenesis is very meager. The luminous organ of *Photinus pyralis*, in common with those of the other *Lampyridæ* which have been studied, consists of two layers of cells, under the outer transparent chitin. These layers of cells are penetrated by numberless tracheæ, the ends of which appear to be connected by a network of very fine tracheoles, the whole system resembling the finer veining of a leaf. On the inner surface of the organ these tracheæ unite to form larger passages. It is practically certain that during the life of the insect these tracheæ are filled with air. Of the two cell layers, the outer consists of a mass of some special type of nucleated cell, of unknown nature, penetrated by the aerophore cylinders, while the inner layer is composed of guanine and sodium or ammonium urate, and probably serves as a reflector. These guanine reflecting layers have also been found in the luminous organs of various fish and cephalopods, whose names I will not attempt to inflict on the readers of this paper. The luminous apparatus of these fish and other of the higher sea-forms is quite highly developed, there frequently being a definite lens with a light-producing body back of it, and behind this a guanine reflector, sometimes with a roughly parabolic section.

It is pretty well established that all photogenic organisms require at least two constant chemical factors in order to exhibit their luminous property, viz., the presence of oxygen and of moisture. Bischoff has claimed that the photogenic process in the luminous rhizomorphs (mycelia) is accompanied by the absorption of oxygen and the evolution of carbon dioxide, and other observers have made similar

^{*} From a paper read before the Chemical Society of Washington, October 13th, 1910.

claims for other cases of biophotogenesis; my own observations tend to support these claims, but as yet the evidence is somewhat too meager, at least so far as the evolution of carbon dioxide is concerned. Dubois's theory assumes the oxidation of a substance of unknown composition, to which he has given the name "Luciferine," through the agency of the oxidase "Luciferase." Until more definite data are at hand, it would seem that this theory requires some caution in acceptance. The facts so far as known certainly present some analogy to other known biologic processes, and it is not at all impossible that his explanation may be correct. Watasé expresses the view that in *Noctiluca miliaris* and other simple luminous forms, the "phosphorescence" is associated with the contractility of protoplasm, as a potential property of all protoplasm, whether exhibited or not, and he rather leaves the reader with the impression that he believes that the particles of food-materials are actually burned in the living tissues with the production of an incandescent temperature. (*Protoplasmic Contractility and Phosphorescence*, Biologic Lectures, Wood's Hole, Mass., 1898.) Jousset de Bellesme, in 1880 (*Comptes Rendus de la Academie des Sciences*, Paris), stated that he believed the light to be due to the spontaneous combustion of phosphine, liberated by the decomposition of protoplasm, and Sir Humphry Davy noted that Lavoister held a similar view. The nature of the substance consumed in this biologic oxidation—the *Noctilucin* of Phipson, the *Luciferine* of Dubois, and the *Photogene* of Molisch—has been variously regarded by different authors. Many seem

to have regarded it as a fat or a fat-like substance; Phipson, who apparently isolated and analyzed a culture of photogenic bacteria, concluded that it contained nitrogen; Kölliker and Macaire believed it to be an albuminous body. Embryologically, it appears to be an extension of the fat-layer. Various observers have found the urates and phosphates of ammonium, sodium, potassium and calcium in the luminous tissue and its ash. Dubois at one time seems to have rejected the oxidation theory and to have believed that the light was due to the spontaneous crystallization of ammonium urate. All attempts to definitely extract and analyze the active substance have failed. When the luminous organs are treated with alcohol or ether in an atmosphere of hydrogen, the liquid acquires a yellow color, but no light-emission occurs when it is exposed to the air or treated with hydrogen peroxide. Lecithin does seem to exist in the insect in small amount.

An interesting circumstance in this connection is the existence in certain luminous organisms of a substance whose solutions exhibit a brilliant blue fluorescence. Dubois found this substance in the cucuyo, and Dr. Coblentz has found it in *Photinus pyralis*, *Photinus corrucus*, and *Photuris pennsylvanica*. I have found it in *Photinus scintillans* also. Dubois regarded the substance he extracted from the cucuyo as analogous to esculin (a glucoside which is present in the bark of the horse-chestnut, and whose solutions possess a blue fluorescence), and he regarded it as essential to the photogenic process, attributing to the actinic properties of the blue rays

in its fluorescent light the actinic power of the emitted light of the insect. Personally, I am inclined to regard the fluorescence simply as an incidental property dependent on the structure of some compound frequently met with in insects of this nature, much as Jordan regards the fluorescent pigment of *Bacillus fluorescens liquefaciens*. Fluorescent extracts of the *pyralis* are produced by extraction with alcohol, ether and water, but not by chloroform, benzene, or carbon tetrachloride. The fluorescent material is not precipitated by lead acetate, mercuric chloride, ammonium sulphate, nor chlorplatinic acid. It appears to be a solid at ordinary temperatures, though as emitted by the insect it is contained in a sticky exudation, which soon hardens in the air.

We cannot say now what possibilities lie before us in the discovery of the "secret of the firefly," particularly as to the kind of "oil" he uses in his little lamp. Perhaps it will be discovered and turned to practical account. The emitted light of the firefly is far from being a good light for general illumination, in spite of its luminous efficiency of 96.5 per cent., on account of the very limited range of color effects possible under it. A single firefly has been variously estimated to give from 1-840 to 1-1600 of a candle power, so we would need quite a high "firefly power" to light our homes and streets by biophotogenic light. There are still many gaps in our knowledge of this interesting subject, in spite of the large amount of work that has already been done, but one by one we hope to close these up and discover the secret of "The Cheapest Form of Light."

The Camphor Industry*

Japanese and Formosa Methods

CAMPION, produced from the camphor tree (*Cinnamomum camphora*) has a large use not only as a drug but more especially in the celluloid industry, where it is used in the transformation of cellulose nitrate to celluloid. It is used extensively in the manufacture of smokeless powder, in the making of substitute leathers, and generally in the plastic pyroxylin industry.

Imports of crude camphor into the United States for the fiscal year ending June 30th, 1910, were 3,026,648 pounds, valued at \$921,926; refined and synthetic camphors added to this 477,269 pounds, valued at \$179,965.

The sources of camphor are practically all foreign. Experimental plantations in the United States will not be ready for production within three or four years, and while the indications are all toward the success of this venture, the commercial production of camphor on a large scale in this country is not to be expected in the immediate future.

At present, barring the production of synthetic camphor, our sources of supply are the East Indian Islands, Borneo, Sumatra, and particularly Japan and Formosa. In Japan and Formosa the camphor trade is a government monopoly. All camphor and camphor oil produced must be sold to the monopoly bureau. The amount of camphor to be placed on the market, the price to the producer, and to the vendee, are fixed by the government.

The privilege of engaging in the industry is granted by the government to individuals and companies on application. The applicant is required to furnish some evidence of his financial standing. A certain territory is allotted to each operator, and he must confine his operations to that tract.

The trees are found only in mountainous districts. In Formosa they are in the interior, where the collection of camphor is rendered hazardous by the presence of the head-hunting tribes which infest the region. Conditions have improved in this respect since the Japanese occupation of the island, owing largely to the conciliatory policy adopted by the Japanese officials, yet in 1909 there were 96 workmen killed by the savages. Owing to the danger of attacks by the tribes wages are high, being 90 cents gold for a native and \$2 for a Japanese.

The condition and extent of the forests have been variously estimated. It is sometimes stated that the camphor forests are inexhaustible. Again, it is estimated that the Formosan forests will be exhausted in twenty years. As a matter of fact the truth is between these limits; perhaps forty-five years of production at the present rate will use up all the trees now workable.

However, the government and private individuals have been planting young trees. About 15,000,000 trees have been set out since 1901, and with a continuance of the policy of yearly planting it is probable that there will always be a sufficient supply of producing trees.

All camphor trees do not produce camphor. In

Borneo, for instance, it is said that only one tree out of 100 bears camphor crystals. The lumber from the trees is valuable, however, and is much used for building, as it is less subject to the ravages of the white ant than most woods. If the camphor odor is quite strong the boards may be used for insect-proof boxes.

In selecting the trees which are to be treated to extract the camphor there is nothing in the outside appearance to guide the searcher. Chips are taken from the trees, and if the camphor odor is absent the tree is not cut. The trees sometimes are 3 or 4 feet in diameter and 180 feet tall. If the odor from the chips is sufficiently strong a search is made over the tree for a flaw in which the camphor gum may have collected. The tree is cut off below the flaw and the wood split away in order to find the deposit. The flaw may be from 4 to 6 feet long and may contain up to 14 pounds, though 7 pounds would be above the average.

In Japan and Formosa the body of the tree which contains camphor is cut into chips by the workers, who use for this purpose a chisel-like knife. The finer the chips the more suitable they are for distillation. They may be taken from trunk, limbs, roots, or twigs. The chips are now distilled in an apparatus of native manufacture which is crude, but better than mechanical devices which have been imported from Europe.

Over a fireplace is a pan filled with water. Above the pan is a very tight circular wooden retort, about 3 feet in diameter at the bottom and 1 foot at the top, and 5 feet high.

This retort is filled through an opening in the top with the camphor wood chips. The opening is then closed by a double lid, and earth banked around the lower section of the retort prevents the escape of the camphor fumes. Just above the water pan are thin slats of perforated bamboo, through the apertures of which steam passes into the chips above, extracting the camphor. Near the top of the retort are two bamboo pipes, through which the vapors are conducted to a cooling box about 10 feet distant. This cooling box is divided into two compartments; the lower is filled with cool water, and the upper contains a wooden screen, upon which the camphor crystallizes. Connected with the cooling box is another of exactly similar description, called the crystallization box. Both are immersed in water, which hastens the process of crystallization. Distillation requires about 24 hours. The chips are then removed through an opening in the lower section of the retort.

The two products of the distillation are crude camphor and camphor oil. The camphor is crystallized on the screens of the boxes. The oil is caught in leads and also skimmed from the water in the cooling boxes.

The camphor and camphor oil thus obtained are shipped to Kobe, Japan, where the camphor oil is further treated. It is heated in small cast iron stills and the fraction having a specific gravity 0.825 to 0.920, which contains 45 to 55 per cent of camphor, is cooled to allow the camphor to crystallize out. When

ready, the crude camphor is drained out of the oil by filtering through cloth bags.

After draining for a few days this product is packed in tubs, and after some months gives by rearrangement, crystals quite as large and firm as those of the crude camphor produced from the wood.

These two grades produced from the wood and from camphor oil when mixed at the monopoly plants constitute what is known as grade "B," or commercial crude camphor. This is used by the American trade.

In the government refineries the "B" grade is somewhat purified, and yields the "BB" grade, which is exported along with the "B" grade to European markets. This is also the grade supplied to the seven companies licensed by the government for refining. They sublime the camphor, producing a clear block of pure camphor.

There are two kinds of camphor on the market—the Japanese $C_{10}H_{16}O$, or ordinary camphor, and Borneo camphor obtained from *Dryobalanops camphora*, $C_{15}H_{24}O$. The latter is more expensive, owing to the demands of the Chinese trade, and is seldom in American or European markets. It can, however, be produced by reduction of ordinary camphor. The odor of Borneo camphor or borneol is much like that of ordinary camphor, but it has in addition a somewhat peppery smell.

The tremendous increase in the use of camphor caused an increase in exports from Japan and Formosa of from 620,000 pounds in 1868 to 8,427,000 in 1907, and at the same time a rise in price of from \$16.42 to \$168.50 per 220 pounds. Owing to the extraordinary prices, industrial chemists were led to investigate the question of producing synthetic camphor, with such success that in 1905 and following they were able to compete with the natural product. In 1908 the effect upon the market of synthetic camphor was such as to reduce imports of natural camphor nearly one-half as compared with 1907.

Immediately following, however, Japan and Formosa were able to quote prices so low that practically all of the firms manufacturing synthetic camphor have been forced to discontinue operations, and natural camphor not only has regained its old market, but is striving with success to find new ones, mainly in the extreme East. It is true, however, that the rapidly increasing price of turpentine may have had something to do with this condition of affairs, and also that in a few cases, notably that of the Chemische Fabrik a. A. vorm. A. Schering of Berlin, it seems possible to make the artificial product and keep its market under present conditions.

Many patents have been taken out covering processes for the formation of synthetic camphor, but all are attempted improvements on the one original discovery—i. e., that pinene, a hydrocarbon in turpentine, could be converted.

The methods for effecting the transformation are various, but all follow practically the same line. Pinene, $C_{10}H_{16}$, is treated with HCl or oxalic acid. In the first case the pinene hydrochloride is heated under pressure with acetate of lead in acetic acid, causing

* From U. S. Consular Report.