Photogenic Substance in the Firefly

Experiments Made to Ascertain How the Insect Produces Light

By E. Newton Harvey

PREVIOUS research on the subject of biophotogenesis has shown that at least three factors are necessary for the production of light, namely, water, oxygen, and a photogenic substance. A fourth factor is probably also involved, an oxidizing enzyme, as in other organic oxidations. Concerning this enzyme nothing is known, at least nothing definite in the case of the firefly. Indeed, Kastle's observations indicate that in the firefly no direct oxidizing enzyme (oxygenase) but small amounts of an indirect oxidizing enzyme (peroxidase) and a catalase are present. (Hygienic Lab., Washington, D. C., *Bull.* 59, 1910, 92.)

The old observation that many luminous tissues can be dried and ground up and will phosphoresce, when water containing oxygen is again added, gives us a simple chemical method of investigating the nature of the photogenic material. The dried material may be extracted with water-free solvents (since the photogen does not oxidize in absence of water), and extracted material as well as the residue from evaporation of the filtrate tested for phosphorescence by adding water. Or the dried material may be extracted with oxygen-free aqueous solvents (since the photogen does not oxidize with light production in absence of oxygen), and filtrate and residue tested as before by admitting oxygen. The first method is satisfactory, and has indicated that a large number of fat solvents will extract nothing from the dried tissue and yet leave the photogenic material unharmed. Indeed, the material may be extracted with boiling ether for twenty-four hours without impairing its power to phosphoresce. Boiling alcohol does destroy the power to phosphoresce, and the nature of its action is discussed below. These results, as well as the previous results of McDermott (*Journ. Am. Chem. Soc.*, 1911, xxxiii., 1791; *Smithsonian Report*, 1911, 345) and Dubois (*Orig. Comm. 8th Intern. Congr. Appl. Chem.*, 1912, xix., 86), using fresh watery material, show that the photogenic substance is not a fat or fat-like body of any kind.

The second method—that of extraction with oxygen-free water solutions—is not satisfactory because the photogenic substance breaks up, or at least loses its power to phosphoresce, on standing in contact with water for any length of time even if no oxygen is present. Many attempts were made to extract the dried material with aqueous solvents, and filter the extract in absence of oxygen before it was recognized that such attempts were futile because of the instability of the photogenic substance in oxygen-free water.

My experiments were begun in the winter of 1913 on firefly material collected at Princeton, N. J., and dried over CaCl_2 in a vacuum. (See preliminary note, Harvey, *Science*, 1914, xl., 33.) I am greatly indebted also to Mr. F. Alex. McDermott, of the Mellon Institute, University of Pittsburgh, for an additional supply of material with which the work was continued. Mr. McDermott has been making experiments along similar lines with a somewhat different apparatus, and his results are likewise published in the *Journal of the American Chemical Society* (*loc. cit.*) As luminous material may be found which does not disintegrate in water the apparatus used for oxygen-free extraction is described below.

The material to be extracted is placed in the vessel C (in the drawing), provided with a ground-in stopper connected with a 120-degree stopcock. The water to be rendered free of oxygen is placed in B after passing hydrogen through stopcock C, and closing it. B is connected through A with a hydrogen generator. The hydrogen is passed through potassium hydroxide to remove acid, and then over a glowing platinum wire (in A) to remove the last traces of oxygen, a much better method than passing the gas through alkaline pyrogallol. By alternately exhausting B through b, connected to an air-pump, and refilling with hydrogen several times the water can be quickly rendered free of oxygen. C is then connected to B through c, and one of the arms of the 120-degree stopcock (d) whose other arm is connected with an air-pump. C and the arms of d are then exhausted. The 120-degree stopcock is then turned to connect C and B, and c is opened, allowing the pressure of the hydrogen to drive the solvent on the material in C. The proper amount of fluid for extraction should be placed in B so that the hydrogen may follow it through and fill the chamber C. Then d is closed, when C can be disconnected and shaken during extraction. To filter the extract it is only necessary to connect one of the arms of d with a desiccator fitted with funnel and filter rack. When the desiccator is exhausted, C and the desiccator are connected, and the pressure of the hydrogen in C

drives the extract onto the filter-paper. The firefly photogen begins to phosphoresce when the atmospheric pressure reaches 5 to 6 millimeters, which means an oxygen pressure of 1 to 1.2 millimeters. Consequently, it is necessary to use a good vacuum pump and make connections air-tight. I found small bore lead tubing sealed with Khotinsky cement the best for the purpose.

If one extracts with distilled water for a short time (fifteen minutes) and then filters, on admitting oxygen the filtrate is found to be dark while the residue on the filter-paper shows the bright points of light characteristic of the power of the firefly. But if the extraction be carried out for an hour or more, neither the filtrate nor the residue will phosphoresce when oxygen is admitted. All of my experiments have been carried out in the dark, and the material observed at critical stages (as when the oxygen-free water was added) to make sure that no light appeared, and always with negative results. But to make sure that no very slow leakage of oxygen into the filtering chamber occurred, I have carried out the extraction in a special tube provided with a capillary sealed off during the extraction. After extracting in this tube for one and one half hours and admitting oxygen no phosphorescence appeared. Thinking that possibly the pho-

togen dissolved in the extracting fluid did phosphoresce, but only so faintly as to be invisible because distributed through a relatively large volume of extract fluid, the unfiltered extract was evaporated *in vacuo* to a small volume. This can be very easily done by placing the rubber tube from the vacuum pump over the capillary onto the special tube, exhausting, and then breaking the capillary through the walls of the rubber tube to connect with the air-pump. Even when concentrated, the extract gave no light on adding oxygen.

The photogen is therefore destroyed in distilled water without oxidation. The search for a watery solvent for photogen becomes then a search for a solvent in which the photogen is stable. The following solutions were tried in addition to distilled water. Extraction was allowed to proceed for from 1 to 1.5 hours.

1. Ringer's solution (as representing fairly accurately the concentration and composition of the firefly's blood).
2. 0.125 M NaCl.
3. Sea-water (a mixture of chlorides and sulphates of Na, K, Ca, and Mg).
4. Five per cent NaCl.
5. 0.05 M NaOH and 0.01 M NaOH. The dried powdered firefly organs will phosphoresce strongly if sprinkled on the surface of 0.1 M NaOH.
6. 0.02 M HCl. Dried firefly powder will phosphoresce on 0.0125 M HCl and on 0.025 M HCl, but less brilliantly. Only one or two bright dots appear on 0.05 M HCl, and no phosphorescence occurs on 0.1 M HCl. If neutralized within two minutes after contact with the acid, the light does not appear in the 0.1 M HCl treated material nor

become brighter in the 0.05 M and 0.025 M material.

In each case after extraction, oxygen was admitted and the solution shaken, yet in no case did light appear either in the undissolved residue or in the solution. The 0.02 M HCl extract was also neutralized as it is well known that the acid prevents biophotogenesis. The conditions of phosphorescence in the firefly are therefore more complex than at first supposed. Either the photogen, the enzymes, the enzyme activators, or all three, undergo changes which are not oxidative in nature, when the material stands in contact with water for a time sufficient to dissolve out the luminous material. Both McDermott's results and mine agree perfectly, and while negative and disappointing they are deemed worthy of publication as indicating that water, oxygen, and a photogenic substance are not the only factors involved in light production, and also as showing the instability of the photogen.

My work with water-free solvents has been confined to those listed in Table 1, which gives also the time of extraction, temperature, and results.

TABLE 1.

Substance.	Temperature, Degrees.	Time, Hours.	Extracted Material.	Extract Evaporated in Vacuo.
Ether (cold).....	20	72	+	—
Ether (hot).....	55	24	+	—
Chloroform (cold).....	20	72	+	—
Chloroform (hot).....	61	8	+	—
Ethyl alcohol (cold).....	20	24	+	—
Ethyl alcohol (hot).....	78.4	24	—	—
Ethyl alcohol and ether (equal parts) boiling.....	44	10	+	—
Carbon tetrachloride.....	20	48	+	—
Carbon disulphide.....	20	48	—	—
Acetone.....	20	48	+	—
Toluol.....	20	48	+	—
Amyl alcohol.....	20	48	Very faint*	—
Ethyl butyrate.....	20	18	Very faint*	—

*The material was washed with ether to remove the amyl alcohol and ethyl butyrate.

A plus sign indicates phosphorescence when water is added, and a minus sign indicates no phosphorescence. Both the original extracted material and the residue of the filtered extract evaporated to dryness were examined. The results indicate that the photogenic substance is not a fat or oil, and also not a lecithin. I am aware that the lecithins are difficult to extract *in toto* from the cell, but this can be accomplished by a mixture of hot ether and alcohol, and yet a mixture of hot ether and alcohol will extract nothing which will phosphoresce from the firefly powder. We may safely say that the photogen is not a lecithin.

Of all the solvents tried only hot alcohol and cold amyl alcohol and ethyl butyrate gave results that would indicate a possible solution of the photogenic substance. And yet there is nothing in the filtrate residue that will phosphoresce when water or a neutralized 3 per cent solution of H_2O_2 is added. Thinking that oxidizing enzymes might be necessary, and that these had not been extracted by the fat solvents although the photogen had, the filtrate was also tested by adding a water extract of firefly organs, fresh or preserved with toluol or chloroform, and also by potato-juice which contains considerable quantities of oxidizing enzymes. In no case was phosphorescence observed. The boiling ethyl alcohol (see Note), cold amyl alcohol, and ethyl butyrate must therefore break up the photogen. It is the alcohol itself and not the temperature (78.4 degrees) of boiling alcohol which is responsible for the destruction of the photogen, as the dried powder will withstand this temperature for twenty-four hours without any appreciable diminution in its power to phosphoresce. McDermott finds that liquid sulphur dioxide and liquid ammonia also destroy the photogenic power.

(NOTE.—The 99.8 per cent absolute alcohol was distilled over metallic calcium and collected in a receiver protected from the air by CaCl_2 in order to remove the last traces of water.)

The powder obtained by drying cultures of luminous bacteria behaves similarly to the firefly material.

These results indicate that it will be a vastly more difficult problem to isolate and identify the photogenic substances than might at first be supposed.—*Journal of the American Chemical Society*.