air is foreign to the natural gas as it occurs in the wells and should, therefore, be eliminated. There is, however, about 1.0% nitrogen and about 0.03% carbon dioxide which are not shown in the analysis as presented.

FRACTIONATION OF NATURAL GAS FROM WHICH GASOLINE IS CONDENSED.

	A. Cc.	B. Per cent.	C. Per cent.
Air + nitrogen	24.5	13.2	....
Methane	59.0	31.9	36.8
Ethane	52.5	28.3	32.6
Propane	34.1	18.4	21.1
Butanes (chiefly)	9.3	5.0	5.8
Pentanes and hexanes	5.9	3.2	3.7
Total	185.3	100.00	100.0

For comparison there is shown the fractionation analysis of the natural gas of Pittsburgh.

FRACTIONATION ANALYSIS OF THE NATURAL GAS OF PITTSBURGH.

Constituents.	Per cent.
Nitrogen	1.6[1]
Methane	84.7
Ethane	9.4
Propane	3.0
Chiefly butane	1.3
Total	100.0

Considerable difference will be noted between the quantity of the different paraffins in this gas mixture and in the one first described. The natural gas of Pittsburgh is used in immense quantities in the east for domestic and other purposes. The other represents a gas used for the condensation of gasoline. Temperatures are in degrees centigrade. Experimental details are shown in a previous communication.[2]

PITTSBURGH, PA.

EXPERIMENTS ON THE NATURE OF THE PHOTOGENIC SUBSTANCE IN THE FIREFLY.

BY E. NEWTON HARVEY.

Received December 19, 1914.

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[3] observations indicate that in the firefly

[1] Includes a trace of carbon dioxide, about 0.03%.

[2] *Loc. cit.*

[3] J. H. Kastle, Hygienic Lab., Washington, D. C., *Bull.* **59**, 92 (1910).

no direct oxidizing enzyme (oxygenase) but only small amounts of an indirect oxidizing enzyme (peroxidase) and a catalase are present.

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[1] and Dubois,[2] using fresh watery material, show conclusively 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.[3]) 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 this journal.[4] As luminous material may be found which does not disintegrate in water the apparatus used for oxygen-free extraction is described below.

[1] F. A. McDermott, THIS JOURNAL, **33**, 1791 (1911); *Smithsonian Report*, **1911**, 345.

[2] *Orig. Comm. 8th Intern. Congr. Appl. Chem.*, **19**, 86 (1912).

[3] *Science*, **40**, 33 (1914).

[4] *Loc. cit.*

The material to be extracted is placed in the vessel C (Fig. 1), provided with a ground-in stopper connected with a 120° 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° stopcock (*d*) whose other arm is connected with an air pump. C and the arms of *d* are then exhausted. The 120° 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 presssure reaches 5–6 mm., which means an oxygen pressure of 1–1.2 mm. Consequently it is necessary to use a good vacuum pump and make connections air tight. I found that small bore lead tubing sealed with Khotinsky cement the best for the purpose.

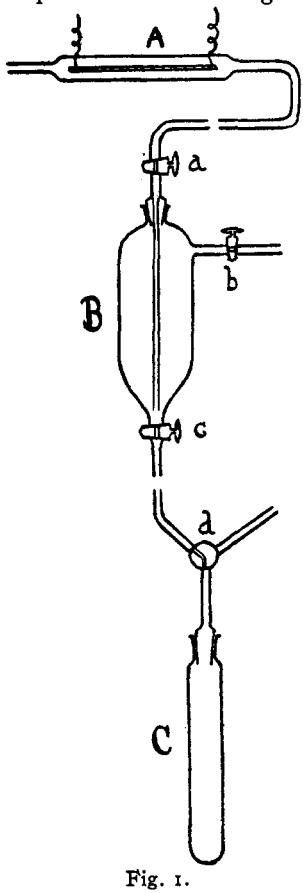

Fig. 1.

If one extracts with distilled water for a short time (15 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 powder 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 photogen 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 the 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 hrs.

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 sulfates of Na, K, Ca and Mg).
4. 5% 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* treated 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 I, which gives also the time of extraction, temperature and results.

TABLE I.

Substance.	Temperature. Degrees.	Time. Hrs.	Extracted material.	Extract evaporated *in vacuo*.
Ether (cold)	20	72	+	—
Ether (hot)	35	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 disulfide	20	48	—	—
Acetone	20	48	+	—
Toluol	20	48	+	—
Amyl alcohol	20	48	very faint[1]	—
Ethyl butyrate	20	48	very faint[1]	—

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% 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,[2] cold amyl alcohol, and ethyl butyrate must, therefore, break up the photogen. It is the alcohol itself and not the temperature (78.4°) of boiling alcohol which is

[1] The material was washed with ether to remove the amyl alcohol and ethyl butyrate.

[2] The 99.8% 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.

responsible for the destruction of the photogen as the dried powder will withstand this temperature for 24 hrs. without any appreciable diminution in its power to phosphoresce. McDermott finds that liquid sulfur dioxide and liquid ammonia also destroy the photogenic power.

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 substance than might at first be supposed.

PRINCETON UNIVERSITY, PRINCETON, N. J.

EXPERIMENTS ON THE NATURE OF THE PHOTOGENIC PROCESSES IN THE LAMPYRIDAE.

BY F. ALEX. MCDERMOTT.

Received December 19, 1914.

During the summer of 1914, the writer made some tests along the same line as described in the preceding paper by Harvey (p. 396), and he also attempted to obtain evidence of the breaking down of nucleic acids during the photogenic process, as suggested by Lund.[1] While the results are mainly negative, they are of interest as confirming Harvey's experiences. The obvious limitations to such work, owing to the restricted amount of material available at one time, is a serious handicap to very extensive results. Most of the present writer's experiments were made on material prepared from *Photinus pyralis* and *P. castus;* some of the material from the former species had been collected at Washington, D. C., in the summer of 1911, and, after drying *in vacuo* over sulfuric acid, had been sealed *in vacuo* in small flasks. This latter material was very kindly supplied to me by Professor J. H. Kastle, Director of the Kentucky Agricultural Experiment Station. It was apparently as active as when first prepared.

Extractions were made in oxygen-free natural gas. After grinding, the tissue to be tested was placed in a small separatory funnel which had been filled with gas which had been passed through pyrogallol solution; the washed gas was allowed to pass through the funnel for some time to sweep out any air which may have entered when the tissue was placed in the funnel. For the filtering, a vacuum desiccator was fitted up with a small beaker, a wire funnel support and a small funnel with a folded filter. The desiccator was connected to the gas supply for some time in order to remove all the air. The solvent to be used was placed in a small Erlenmeyer flask provided with a tightly fitting stopper bearing two small bore stopcocks. The washed gas was passed through this flask for some time after the solvent had been run in, and the solvent was then heated

[1] Lund, *J. Exper. Zoöl.*, 11, 415 (1911).