STUDIES ON BIOLUMINESCENCE.

XI. Heat Production during Luminescence of Cypridina Luciferin.

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Dubois[1] obtained some evidence of infra-red radiation given off during the flash of the West Indian firefly, *Pyrophorus*. Langley and Very[2] also at first observed a slight heating of their bolometer from the light of the same insect, but in a later investigation, Langley[3] was unable to detect any heating of his bolometer whatsoever. Coblentz,[4] using a vacuum thermopyle, could detect no infra-red radiation from the American firefly, *Photinus*. Ives[5] also, using a phosphorphotographic method, found no infra-red radiation in the light of *Photinus*. We must attribute the earlier evidence to experimental errors and conclude that the light of luminous animals contains no rays of wave-length longer than the visible.

This does not mean, however, that no heat is produced by the reaction which produces the luminescence. A temperature change of a few thousandths or hundredths of a degree would evolve no measurable radiation. Coblentz[4] first studied the problem of heat production in the firefly, using a thermo-couple as the measuring instrument. He came to the conclusion that the temperature of the insect was slightly lower than the temperature of the air and that the luminous segments were slightly hotter than the non-lumi-

[1] Dubois, R., *Bull. Soc. Zool. France*, 1886, xi, 1.

[2] Langley, S. P., and Very, F. W., *Am. J. Sc.*, 1890, xl, series 3, 97.

[3] Langley, S. P., *Am. Astrophys. Obs.*, 1902, ii, 5.

[4] Coblentz, W. W., *Carnegie Institution of Washington, Publication No. 164*, 1912.

[5] Ives, H. E., *Phys. Rev.*, 1910, xxxi, 637.

nous segments. No definite increase or decrease in temperature could be established during the flash of the firefly. However, further work on the firefly is much to be desired.

The use of a living animal for such measurements introduces a possible source of error, in that any contraction of the muscles of the animal will produce heat which may add to an increase, or mask a decrease of temperature during luminescence. Utilization of extracts of luminous animals avoids the complications due to muscular contraction. From *Cypridina hilgendorfii*, a small crustacean, may be prepared a solution of luciferin, an oxidizable substance, and a solution of luciferase, a catalyzer which accelerates the oxidation of luciferin with light production. By bringing the solutions of luciferin and luciferase to the same temperature and then mixing them, one can measure any increase or decrease of temperature which occurs during the luminescence resulting from mixing. We can thus gain some idea of the heat of oxidation of luciferin.

Although the experiment sounds very simple it is actually somewhat difficult to carry out. The attainment of temperature equilibrium between two solutions is very slow when one wishes to obtain it to within 0.001°C. of the same temperature. After many attempts, the following arrangement of apparatus (Fig. 1) was found most satisfactory. About 10 cc. of luciferin solution were placed in the inner tube (D) of a special non-silvered thermos bottle (A). About 1 cc. of luciferase solution was placed in a very thin-walled glass tube (E) which was immersed in the luciferase solution and connected with a small motor so that it could be slowly but constantly rotated, thus stirring the solutions. Thermo-couples (L and M) of advance (0.008 inch) copper (No. 30, B and S enamel-insulated) wire were paraffined and placed in each tube and the copper wires connected through a copper double throw switch (C) with a Leeds and Northrup d'Arsonval wall galvanometer (No. 34,637, silver strip suspension) of 35 ohms resistance and 310 megohms sensitivity. The constant temperature junctions (N) were placed in a large Dewar flask (B) and filled with water at approximately the same temperature as the luciferin solution. 1 mm. galvanometer scale division represented 0.003°C. and the division readings could be estimated to tenths. By means of a glass rod (F) placed in the tube containing luciferase solution,

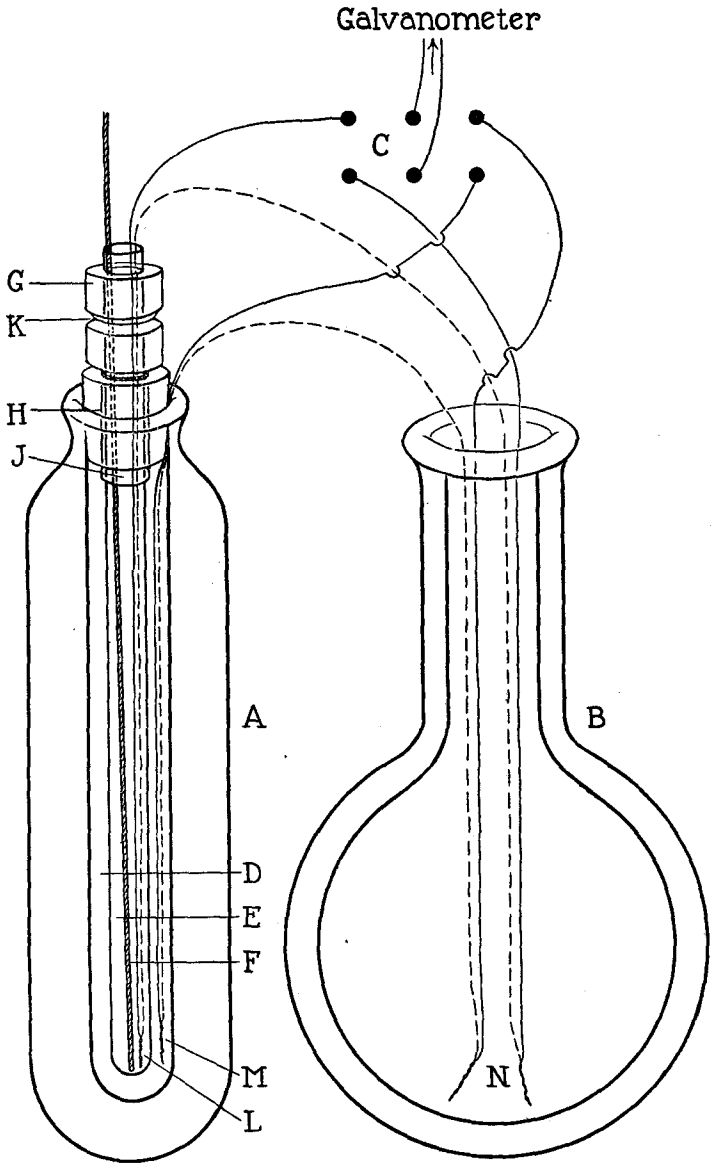

FIG. 1. Apparatus for determining heat production during luminescence of luciferin. A, special thermos tube. B, Dewar flask for constant temperature junctions. C, copper double throw switch. D, tube containing luciferin solution. E, tube containing luciferase solution. F, glass rod for breaking E. G, rubber stopper with groove, K, for pulley cord. H, cork closing thermos tube. J, brass sleeve in H allowing rotation of E. L, thermo-couple in luciferase solution. M, thermo-couple in luciferin solution. N, constant temperature junctions.

this tube could be broken and the luciferase and luciferin solutions mixed.

It was found that even after the luciferase and luciferin solutions came to the same temperature within the thermos bottle, this was not necessarily the same as that of the room, and a slow rise or fall occurred as indicated by a slow drift of the galvanometer coil. Readings of each thermo-couple on the galvanometer scale were therefore taken at 1 minute intervals for some time before and after mixing the luciferin and luciferase solutions and plotted as curves. Control experiments were also carried out in exactly the same manner as the luciferin-luciferase experiments but water was placed in the two tubes instead of luciferin and luciferase. Figs. 2 and 3 give typical experiments with water and with luminescent solutions respectively. As can be seen from the curves, the rise in temperature in each case figured is 0.006°C., or two scale divisions.

With both control (water) and luciferin experiments there was a slight rise in temperature on mixing the liquids in the two tubes. The average rise of five control (water) experiments was 0.0054°C. and the average rise of five luciferin experiments was 0.0048°C.

In one control experiment there was no change in temperature on mixing and in one luciferin experiment there was a slight drop in temperature (0.0045°C.) on mixing. The average rise in temperature is no doubt due to heat from friction in the mixing of the liquids and the breaking of the glass tube. The difference in the average rise of control and of luciferin experiments is so small (0.0006°C.) as to have little significance. We may therefore conclude that if any temperature change occurs during the luminescent reaction it is certainly less than 0.001°C. and probably less than 0.0005°C.; too small to be measured by this method.

To prepare the luciferin solution, 2 gm. of dried *Cypridina* were dissolved in 20 cc. of hot water and 10 cc. of this 10 per cent solution were used in the thermos bottle in the above experiments. If we assume that 1 per cent of the dried *Cypridina* is luciferin, 0.1 gm. of luciferin on oxidation was not able to change the temperature of the 10 cc. (in reality 11 cc., since 1 cc. of luciferase solution was mixed with the 10 cc. of luciferin) of solution 0.001°C. This means that 1 gm. of luciferin liberates less than 0.1 calorie during the luminescence accompanying oxidation.

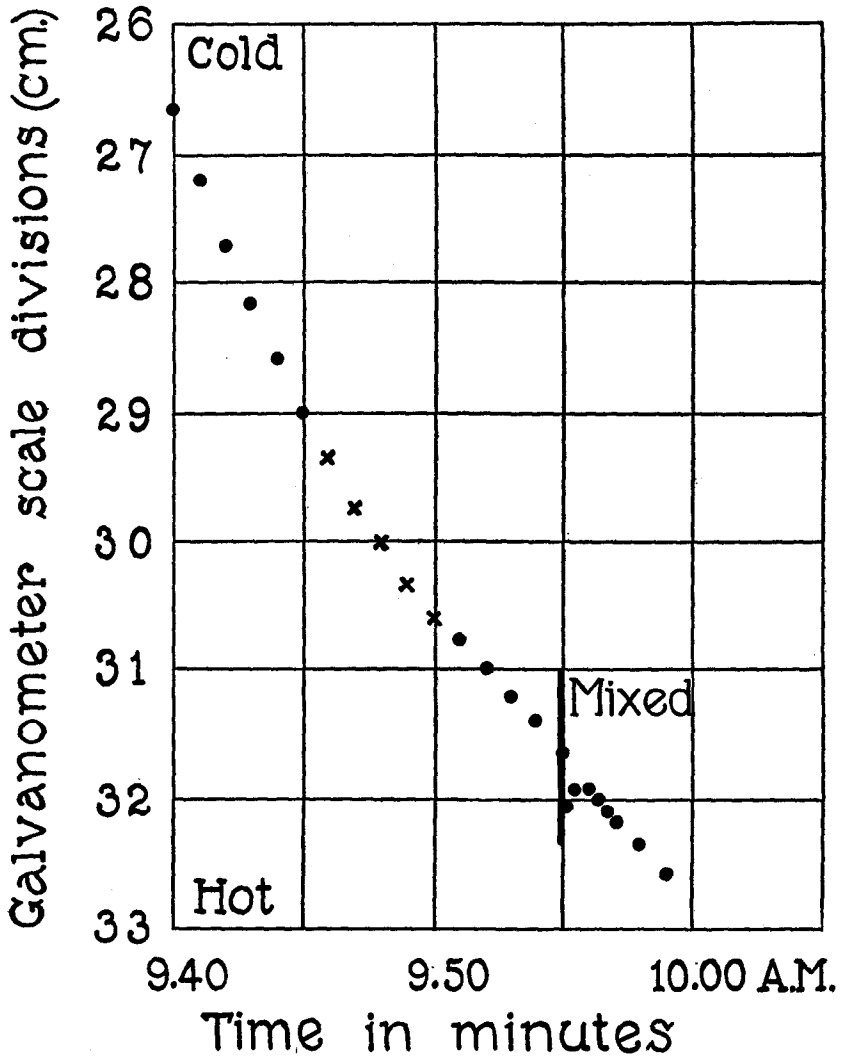

FIG. 2. Curve showing temperature change when two tubes containing water at the same temperature are mixed. 0.1 galvanometer scale division = 0.003°C. Dots represent readings of thermo-couple in tube D; crosses, readings of thermo-couple in tube E.

Since 1 gm. of glucose liberates 4,000 calories on complete oxidation to CO_2 and H_2O, it will be seen that the oxidation of luciferin is a very different type of reaction from the oxidation of glucose. As I have already suggested it is similar to the oxidation of reduced hemoglobin or the oxidation of leucomethylene blue to methylene blue. According to Barcroft and Hill[6] 1.85 calories are produced per gm. of hemoglobin oxidized. I have been unable to find figures for the

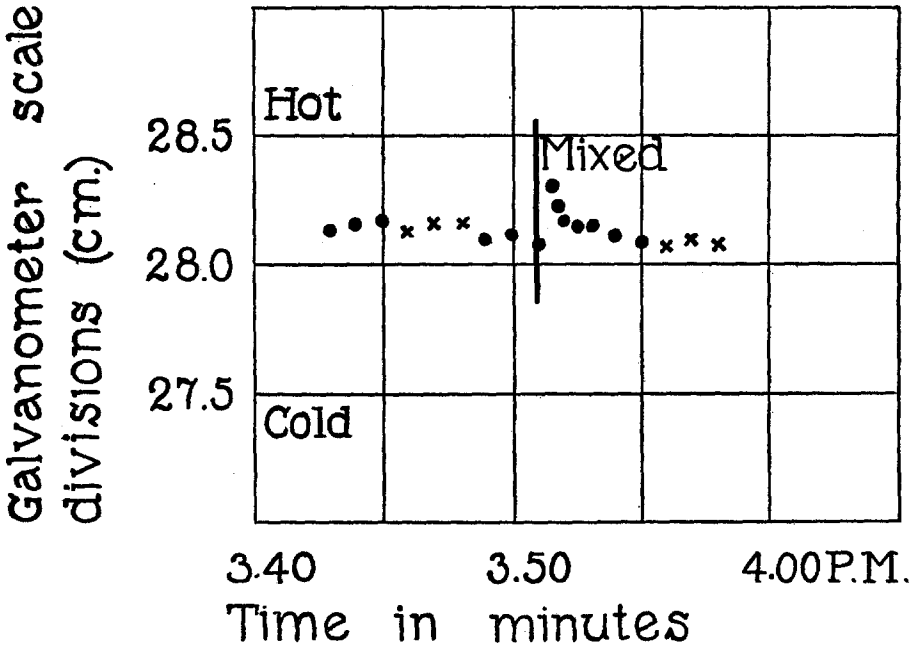

FIG. 3. Curve showing temperature change when luciferin and luciferase solutions at the same temperature are mixed. 0.1 galvanometer scale division =0.003°C. Dots represent readings of thermo-couple in luciferin solution; crosses, readings of thermo-couple in luciferase solution.

heat exchange during oxidation of leuco dyes, but it is no doubt also small.

The production of carbon dioxide always involves the evolution of considerable heat. Since luciferin evolves no measurable amount

[6] Barcroft, M. A., and Hill, A. V., *J. Physiol.*, 1909–10, xxxix, 411.

of heat on oxidation we have further evidence in support of that presented in a previous paper, that no carbon dioxide is produced during luminescence of luciferin. The energy change involved is very small indeed. It is on first thought surprising that so bright a luminescence as that of *Cypridina* should result from a reaction involving only a very small amount of heat. If we recall, however, that the eye is an extraordinarily sensitive instrument (responding to 10^{-9} ergs per second), which can detect light so weak, that, if it were converted into heat, would take 60,000,000 years to raise 1 gm. of water 1°C., we may realize that, after all, a very small heat production during oxidation of luciferin may be sufficiently great to account for its luminescence.

It gives me pleasure to acknowledge the kindly interest and assistance in setting up apparatus of Dr. W. Weniger of the Nela Research Laboratory.