ON THE QUANTA OF LIGHT PRODUCED AND THE MOLECULES OF OXYGEN UTILIZED DURING CYPRIDINA LUMINESCENCE.

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The primary effect in a photochemical reaction is the absorption of a quantum \((h\nu)\) of light energy with the conversion of a single molecule into reaction products. Later, secondary effects, such as the production of a catalyst by the light, may obscure the quantum relation, resulting in much greater chemical change than 1 quantum per molecule. On the contrary, not every quantum of light absorbed by a solution may result in chemical change, but there may be conversion into heat. In this case several quanta per molecule will be absorbed. As a matter of fact photochemical reactions are few in which the ratio of quanta absorbed to molecules decomposed is 1 (Taylor (1)).

In the reverse process, the emission of light through chemical change, chemiluminescence, we may also suppose as the primary process that 1 molecule undergoing change emits 1 quantum of light energy. Is light an invariable accompaniment of each molecular change? The few studies of chemiluminescence from this point of view show that change in many molecules occurs before 1 quantum is emitted. Thus, Haber and Zisch (2), studying the reaction of Na vapor and Cl at low pressures, observed the quanta of sodium D line emitted to be far less than the number of molecules of NaCl formed. This means that of the collisions between Na and Cl resulting in NaCl formation only a few emit light. It is obvious that this is an important point and should be studied further.

The present paper reports the results of an attempt to determine if a molecule of Cypridina luciferin, the oxidizable substance of the

1 A quantum of light is the constant, \(h\), equivalent to \(6.554 \times 10^{-27}\) erg. sec., times the frequency, \(\nu\), of the light in question.
luminous crustacean, *Cypridina hilgendorfii*, emits 1 light quantum on oxidation in presence of luciferase. As luciferin cannot be obtained pure and consequently weighed out with accuracy, it is necessary to measure the oxygen rather than the luciferin used up during luminescence and to assume an oxidative dehydrogenation of luciferin, for which there is considerable evidence, thus:

\[
\text{luciferin} + \frac{1}{2} \text{O}_2 \rightarrow \text{oxyluciferin} + \text{H}_2\text{O}.
\]

From this equation, which accounts for luminescence if luciferase is present, it is evident that 16 gm. or \(3.03 \times 10^{-3}\) molecules of oxygen will oxidize a molecular weight of luciferin. If we measure the amount of oxygen consumed and the total amount of light produced by a given weight of *Cypridina*, the mg. of oxygen per lumen or the molecules of oxygen per quantum can be stated.

The total light produced is measured by direct photometric comparison of the brightness of a given depth of luminescent *Cypridina* solution with a surface whose brightness can be varied by known amounts. As the brightness of *Cypridina* luminescence decays with time, a curve of brightness against time is drawn, whose area will give the total amount of light produced in lumens, after allowing for absorption of light in the yellowish solutions. Details of the measurement are given by Stevens, whose work shows that, with the same amount of luciferin, the total light is somewhat greater with smaller concentrations of luciferase. The total light is about proportional to the mass of luciferin but varies with other factors so that no great accuracy is claimed, even for samples of the same dried *Cypridina* material. In fact, only the order of magnitude of the oxygen per lumen can be given, since measurement of oxygen consumption presents the greatest difficulties.

The difficulty in measuring oxygen consumption by differential manometer methods is due to the fact that luciferin solution is prepared by making a boiling water extract (which destroys luciferase) of dried *Cypridina* and cooling the extract quickly. Such a solution is not in equilibrium with any known pressure of oxygen and cannot be brought into equilibrium with air without the oxidation of a consider-

\[\text{See preceding paper of this journal.}\]
able and unknown amount of luciferin without luminescence. It is therefore necessary to keep luciferin solution away from oxygen until ready to measure its oxygen consumption. The method finally adopted is to allow the luciferin luminescence to indicate the complete oxidation of the luciferin, as explained in connection with Fig. 1.

In Vessel B, of 25 cc. capacity, 4 cc. luciferase is placed (1 per cent dried *Cypridina* solution) and 0.5 cc. mercury to serve as a stirrer. With Cocks F, C and D open, and E closed, a stream of hydrogen freed of oxygen by passage over red hot platinized asbestos in a quartz tube is passed through the vessel for 1 hour, thus removing all oxygen. The hydrogen passes through lead tubing (since rubber tubing

![Diagram of apparatus](image)

is permeable to oxygen) sealed to the F end of the apparatus with de Kohtinsky cement. Cocks C and D are then closed.

A solution of luciferin is then made by extracting 2 gm. of dry *Cypridina* with 50 cc. hot water (4 per cent solution) and immediately decanting into Vessel A. With Cocks F and E open, a stream of pure hydrogen is now passed through A for 1 hour, when E is closed. By opening C and D carefully, luciferin is now mixed with luciferase and B completely filled with fluid, when all cocks are closed and the vessel disconnected from the supply of hydrogen. There will be no luminescence in Vessel B since oxygen is absent.

The remaining luciferin in Vessel A can now be taken to the pho-
tometer bench, mixed with luciferase in presence of oxygen and the total light produced measured as described. In a typical satisfactory experiment it amounted to 0.101 lumens per gm. of dried *Cypridina* material.

Vessel *A* is then filled with distilled water saturated with air at 22°C. Each cc. of distilled water will dissolve 0.006 cc. oxygen (measured at N. T. P.) By opening Cocks *C* and *D*, small amounts (0.1 to 0.2 cc.) of water are allowed to flow into *B*, displacing a corresponding amount of luminescent solution into *G* which can then be measured in *G*. After each admission the fluid in *B* is thoroughly mixed by shaking the mercury back and forth and luminescence noted. Luminescence following each admission of oxygen becomes less and less bright and is quite faint after 1 cc. water has been added, making allowance for the 0.1 cc. capacity of the bore of Stop-cock *C*, which contained no oxygen.

In a typical experiment 1.1 cc. water was admitted before luminescence ceased. To calculate the gm. of oxygen admitted: 1.1 cc. × 0.006 = 0.0066 cc. × 0.00143 = 9.44 × 10⁻⁴ gm. of oxygen were necessary to oxidize the luciferin in 20.5 cc. solution containing 4 per cent or 0.82 gm. dry *Cypridina*. Hence 9.44/0.82 = 11.5 × 10⁻⁶ gm. of oxygen were used per gm. of dry *Cypridina* material. The total lumens per gm. of dry *Cypridina* material was found to be 0.101 in this experiment, as stated above. Hence 11.5 × 10⁻⁶/0.101 = 11.4 × 10⁻⁶ gm. oxygen per lumen.

It is quite obvious that this method of determining oxygen cannot give very accurate results since the end-point is not very sharp. It is also certain that when the admission of oxygen results in only faint luminescence there must be an excess of oxygen present. This is because luminescence intensity is really dependent on reaction velocity and not on concentration of luciferin (Amberson (3)). With considerable luciferin and little oxygen, luminescence will be faint because the low oxygen concentration is slowing reaction velocity; with little luciferin and considerable oxygen, luminescence will be faint because the low luciferin concentration is slowing the reaction velocity. The latter statement corresponds to the condition when we are nearing the end-point in admitting oxygen. In fact it is possible to show that

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3 I am deeply indebted to Mr. K. P. Stevens for making these measurements.
after 1 cc. of water has been admitted to Vessel B, there is excess oxygen present, since fresh oxygen-free luciferin solution introduced into B will give a fair luminescence.

The results tell us only that less than $11.4 \times 10^{-5}$ gm. oxygen per lumen are necessary for a lumen of luminescence. It will be noted also that the assumption is made that all the oxygen admitted to the luciferin solution is used in oxidizing luciferin, and that there are no other easily oxidizable compounds present. I have endeavored to test this point by adding to the dried Cypridina powder an equal weight of dried powdered pill-bugs (Oniscus) which might supply easily oxidizable substances but not luciferin, and then determining oxygen consumption. The runs with added dried Oniscus showed about 25 per cent more oxygen consumed, so that this factor can play no very great part in the oxygen consumption. However, the most that can be claimed is a determination of the order of magnitude of oxygen consumption per lumen of light emitted.

The average of eleven experiments completed without mishap was $10.6 \times 10^{-5}$ gm. of oxygen per lumen, with extreme variations of $5 \times 10^{-4}$ and $15.5 \times 10^{-5}$ gm. per lumen. The average of five of the later more satisfactory experiments was $11.7 \times 10^{-5}$ gm. per lumen with a maximum variation from the mean of 25 per cent.

Upon the basis of $11.4 \times 10^{-5}$ gm. of oxygen per lumen of luminescence, we may calculate the molecules of oxygen per quantum with the aid of the visibility of radiation curve. The energy distribution in the Cypridina luminescence spectrum has recently been determined by Coblentz and Hughes (4). The maximum emission is at $\lambda = 0.48\mu$ and the visibility curve tells us that 1 lumen of $\lambda = 0.48\mu$ light is equivalent to 0.01 watt or $0.01 \times 10^7$ ergs per second. A quantum $(hv)$ of $\lambda = 0.48\mu$ light is equal to $4.1 \times 10^{-12}$ ergs so that 1 lumen of $\lambda = 0.48\mu$ light contains $2.45 \times 10^{16}$ quanta. As $11.4 \times 10^{-5}$ gm. of oxygen contain $2.16 \times 10^{18}$ molecules and will oxidize $4.32 \times 10^{18}$ molecules of luciferin we see that 88 molecules of oxygen or 176 molecules of luciferin must undergo change in order to produce 1 quantum of light of $\lambda = 0.48\mu$.

For reasons stated above the actual value is probably somewhat less than this. A conservative statement might be that about 50 mole-

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4 See data on light units, Trans. Illuminating Engineering Soc., 1922.
cules of oxygen or 100 molecules of luciferin react to produce 1 quan-
tum of Cypridina luminescence. Certainly more than 1 molecule of
oxygen per quantum is necessary.

Accepting 50 molecules of oxygen per quantum or $6.48 \times 10^{-3}$ gm.
per lumen of luminescence, a few other orders of magnitude can be
calculated, based on the luminescence equations (Harvey (5)):

\[
luciferin (LH_2) = \text{oxyluciferin} (L) + H_2 - 15 \text{ Cal.}
\]

\[
H_2 + \frac{1}{2} O_2 = H_2O \text{ (liquid)} + 69 \text{ Cal.}
\]

The heat of oxidation of luciferin is therefore 54 Calories per gm.
mol, justified on the general rule for heats of combustion of organic
compounds (Thornton (6)). The gm. molecular heat equivalent of
the quantum of $\lambda = 0.48\mu$ light is 59.3 Calories, obtained from the
relation $U$ (in Calories) = $N$ (Avogadro constant) $h\nu$, but we are as yet
uncertain whether this relationship can be applied to chemilumines-
cent reactions.

If 16 gm. of oxygen oxidize luciferin with production of 54,000
calories of heat, $6.48 \times 10^{-3}$ gm. of oxygen will evolve $2.19 \times 10^{-1}$
calories and correspond to the emission of 1 lumen of $\lambda = 0.48\mu$ light,
equivalent to $2.39 \times 10^{-3}$ calories. Hence the efficiency, energy in
light/heat of reaction, is about 1.1 per cent. The rise in temperature
in a 4 per cent solution of dried Cypridina should be in the neighbor-
hood of 0.001°C.

The oxygen necessary to combine with the luciferin in 1 cc. of a
4 per cent solution of dried Cypridina, on the basis of 50 molecules per
quantum, is approximately $0.3 \times 10^{-4}$ gm. or a 0.00001 molecular
solution. A 4 per cent Cypridina solution must therefore contain
luciferin in approximately 0.00002 m concentration.

The results show beyond any doubt that more than 1 molecule of
luciferin must react to produce 1 quantum. Luminescence is not
therefore an invariable accompaniment of luciferin oxidation. This
is to be expected if luciferase is the source of the light, which results
from transfer of the energy of oxidation of luciferin to some of the
luciferase molecules, exciting them to luminescence (Kautsky and
Zocher (7)). Spontaneous oxidation of luciferin is proceeding and
only those molecules of oxidizing luciferin can transfer their energy
which occupy special positions as regards the luciferase molecules.
SUMMARY.

A study of the oxygen consumed per lumen of luminescence during oxidation of *Cypridina* luciferin in presence of luciferase, gives $11.4 \times 10^{-8}$ gm. oxygen per lumen or 88 molecules per quantum of $\lambda = 0.48\mu$, the maximum in the *Cypridina* luminescence spectrum. For reasons given in the text, the actual value is probably somewhat less than this, perhaps of the order of $6.48 \times 10^{-8}$ gm. per lumen or 50 molecules of oxygen and 100 molecules of luciferin per quantum. It is quite certain that more than 1 molecule per quantum must react.

On the basis of a reaction of the type:

$$\text{luciferin} + \frac{1}{2} O_2 = \text{oxyluciferin} + H_2O + 54 \text{ Cal.},$$

it is calculated that the total efficiency of the luminescent process, energy in luminescence/heat of reaction, is about 1 per cent; and that a luciferin solution containing 4 per cent of dried *Cypridina* material should rise in temperature about 0.001°C during luminescence, and contain luciferin in approximately 0.00002 molecular concentration.

BIBLIOGRAPHY.

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