STUDIES ON LUMINOUS BACTERIA.

II. THE INFLUENCE OF TEMPERATURE ON THE INTENSITY OF THE LIGHT OF LUMINOUS BACTERIA.*

BY THOMAS F. MORRISON.

(From the Physiological Laboratory of Princeton University, Princeton, and the Nela Research Laboratories, Cleveland.)

(Accepted for publication, May 4, 1925.)

The fact that temperature plays a large part in the production of light by luminous bacteria has been noted by the majority of observers who have identified new forms since Pflüger described the first form in 1875. In practically all of these descriptions there have appeared the temperature limits at which the bacteria might live, and side by side with these are given the temperature limits for the production of the light. However, nothing has been recorded of the relation which exists between the temperature and the brightness of the light, the former reports being confined entirely to the limits at which the process might be carried on. The purpose of this paper is to show the relation existing between the temperature and the intensity of the light of luminous bacteria.

The minimum temperature for light production in the bacteria is, as a rule, very low (in some cases about −20°C.), due perhaps to the fact that the organisms are of such minute size that the substances within them are contained in capillary spaces, a condition tending to lower the freezing point. Whereas a few degrees rise in temperature above that of the optimum for the production of light rapidly brings a complete loss of the photogenic function and the death of the cell, the temperature of liquid air can be tolerated by the bacteria for a period of several hours (Macfadyen), the light

* This is the second of a series of papers which the writer intends to publish on the reactions of luminous bacteria to varying conditions of environment. The first paper was published in collaboration with Dr. E. N. Harvey in J. Gen. Physiol., 1923–24, vi, 13.
returning when the bacteria are again brought to ordinary temperatures. Since the literature on the temperature limits for luminous bacteria is too extensive to be incorporated in a paper of this nature, the reader is referred for a treatment to Harvey's book, "The nature of animal light."

Beijerinck, who has studied many forms of luminous bacteria, finds that the luminescence ceases with death of the organism and that the luminescence is independent of the respiration. He is of the opinion that the luminescence is produced at the moment of the conversion of peptone into living protoplasm. Later observations by B. Fischer, Forster, Lehmann, Tollhausen, and others upon the production of light at or below 0°C., a point too low for growth, hardly seem to agree with this view of the vital nature of luminescence. Still later observers, notably Dubois, and Harvey (b), find that in some forms other than bacteria the production of light is due to the oxidation of a protein-like substrate, *luciferin*, in the presence of an enzyme, *luciferase*.

Although these two substances have been found to be the active elements in several forms, yet one of these, luciferin, has not been definitely isolated from the bacteria. In 1920 Gerretsen published a paper on the reaction of luminous bacteria to ultra-violet light. In this paper, aside from his observations on the effect of the radiations, he describes the method of isolation of the enzyme luciferase, and is led to the conclusion that the substance is of the same nature as the luciferase in other forms. However, thus far the presence of the substrate has not been definitely proven. Although luminous bacteria which have been thoroughly ground up and dried will give a faint luminescence when mixed with fire-fly luciferase (Harvey, c), there are other substances, such as the non-luminous portions of the fire-fly, which will give the same result. It is highly probable that in the bacterial cell the luciferin is in some very unstable form which prevents its isolation, spontaneous oxidation taking place as soon as it comes in contact with the air. Regardless of the fact that there is no absolute proof for the presence of luciferin in the bacteria, I do not believe that it would be amiss to consider the phenomenon of light production as being due to the presence of both of these substances. In view of the work which has been done on the pro-
duction of light by other forms, it is almost impossible to make an exception of these organisms. Therefore, in this work the fundamental assumption is made that the process is enzymic in nature.

_Apparatus and Methods._

The photometer bench designed by Hyde and Cady and used in the Nela Research Laboratories was used as the foundation for the apparatus. Near one end of the bench was mounted a 6-8 volt 21 c.p. auto headlight lamp through which the current could be varied by means of rheostats controlled by the observer. An ammeter was placed in series with the lamp and read by an assistant in an adjoining room.

Next to the lamp was placed a black plush screen of the usual photometric design. Across the opening of the screen were fastened two filters: a neutral gray, 10 per cent Wratten filter, and a mercury green (No. 62) Wratten filter. The purpose of the former was to permit the lamp to be run at a sufficiently high temperature to reduce the color differences of the filament, and yet to bring the intensity of the light down to that of the bacteria. The latter served to match the color of the lamp with that of the light of the bacteria.

In front of the screen was placed a diffusing surface, and in front of that a comparison cube. This comparison cube consisted of two 90° angle prisms cemented together along their hypothenuses with their adjacent sides silvered in bands, in such a manner that a beam of light passing at right angles to the observer's line of vision would be reflected toward him, while a source of light directly in front of him would be transmitted uninterruptedly to him. The two sources of light would then appear as parallel bands of light, a band of transmitted light being bounded above and below by two equally broad bands of reflected light. The comparatively large surface covered by these two bands of light greatly facilitates comparing the intensities of weak lights of the same color.

On the opposite side of the cube from the observer was placed a container for the bacteria. This container consisted of two parts: a small, rectangular glass bottle (inside width 39 mm.) and a surrounding metal water bath. In one side of the water bath a hole had been cut in which a piece of plain glass was mounted, forming a
window. The bottle, containing the bacteria suspended in a balanced salt solution, was placed next to the window so that the light which the bacteria emitted could be observed through it.

A copious supply of air was bubbled through the suspension at all times in order to obviate any possibility of a decrease in the intensity of the light due to insufficient supply of oxygen. Likewise, an even distribution of the temperature in the water bath was insured by passing a stream of air through the water.

A centigrade thermometer, graduated in fifths of degrees, was placed in the bottle containing the bacteria and all of the readings made on it.

The comparison lamp was measured in terms of millilamberts\(^1\) by means of a Macbeth illuminometer. Measurements were made from the side of the cube toward the observer and then plotted as a function of the current through the lamp. The absorption of the screens was also measured at varying temperatures of the lamp and the percentage transmission taken into account when the results were tabulated.

The species of bacteria used in these experiments was *Bacterium phosphorescens*, isolated from fish obtained at the Princeton Fish Market during the fall of 1923 and identified by means of Gotham's chart after Dahlgren. The light of this form is yellow-green in color, the most intense part of the spectrum lying in the neighborhood of 510 \(\mu\)m. For experimental purposes the bacteria were grown on large enamel pie plates, the organisms being scraped from the medium and then suspended in a balanced salt solution of \(pH\) of approximately 7.7 and isotonic with sea water.

A typical experiment would be carried out in the following manner: after the bacteria had been scraped from the medium and suspended in the salt solution, they were placed in the bottle which in turn was placed in the water bath and the entire piece mounted on the photometer. The temperature of the water in the bath was lowered

\(^1\) The lambert is the unit of brightness. When a surface, obeying Lambert's cosine law, emits or reflects one lumen per sq. cm. its brightness is said to be one lambert. For the definitions of other physical light units, the reader is referred to the report published by the Illuminating Engineering Society, New York, N. Y., 1922.
by the addition of ice until it had brought the temperature of the suspension to such a point that the intensity of the light could just be read with ease on the comparison cube. This point was approximately 2°C. A comparison would then be made and the observer would call out the temperature to the assistant in the next room who would then read the ammeter and tabulate the data. When the temperature had been lowered to the desired point and a comparison made, some ice would be removed from the bath and the temperature allowed to rise slowly, readings being taken at as frequent intervals as possible during the rise and the results tabulated. As the temperature approached that of the room it was found that the rate of increase was considerably slowed down. To eliminate the error due to keeping the bacteria at one temperature for too long a time, a low flame was placed under the water bath and the water slowly heated, the higher temperatures being obtained by raising the flame. During the course of one experiment, lasting about 2 hours, as many as sixty values might be tabulated.

RESULTS.

In luminous bacteria one finds a happy combination for a study of this nature. Not only are they of interest from the point of view of their light production, but also one finds here an organism in which energy is emitted in an easily measurable form, and, when taken as an index of a vital phenomenon, can be subjected to a great variety of environmental changes, and a study made of the effects of these changes on the rate of reaction.

Both at excessively high and at very low temperatures the light emitted by the organisms appears yellower in color than that around the optimum temperature. Whether there is an actual change in the quality of the light, or whether this is an example of the Purkinje phenomenon, has not been determined as yet, due mainly to the difficulties encountered in attempting a spectroscopic examination of lights of such low intensities. However, Harvey (d) has noted a similar change in the color of the light of the fire-fly and of ostracods, but only at high temperatures, and he has come to the conclusion that the change is a real one and not subjective.

In Fig. 1 the actual points determined during the course of one
experiment are shown. Here the intensity of the light is plotted along the ordinate as a function of the temperature, plotted along the abscissa. The form of the curve is similar to that obtained for other processes in which temperature is one of the variables.

It will be noted that in the neighborhood of the maximum there are points which lie at some distance from the smoothed curve. At first this was thought to have a special significance, and as a result several experiments were performed to see if there were any changes in the intensity of the light at these points. The temperature in these experiments was maintained at a constant value over a period of 2 to 3 hours; the result of such an experiment is shown in Fig. 2.

It will be seen that the variations which appear in Fig. 1 lie well within the limit of error of setting. To my mind no other explanation of these variations can be given. In calibrating the comparison lamp in terms of photometric units against the current which passes through it, one obtains a curve which rises quite rapidly at

![Graph showing the relation between brightness and temperature.](https://example.com/graph.png)
first, but which gradually approaches a line running parallel to the abscissa as the higher intensities are reached. It is easily seen that when one reads the difference in current, which is plotted along the ordinate, the lower values will not give a very great difference in millilamberts, which are plotted along the abscissa, while a corresponding small difference in current at a higher value will give far larger differences in intensity. Thus a difference between 350 and

![Graph showing brightness over time](graph.png)

**Fig. 2.** Curve showing how the brightness of a bacterial emulsion varies with time. Note that the brightness is practically uniform for 2 hours.

352 milliamperes will give a difference in intensity of only 2 millilamberts, while a corresponding difference between 450 and 452 milliamperes will give a difference in intensity of 11 millilamberts.

In common with a good many physiological processes the production of light has a temperature coefficient which does not follow the rule of temperature coefficients laid down by van't Hoff. From the data furnished by this experiment, the $Q_{10}$ for the process was calculated from the van't Hoff formula as modified by Synder:
Substitution of the values from the experiment in this equation gives the following list of values for $Q_{10}$.

<table>
<thead>
<tr>
<th>Temperature interval</th>
<th>$Q_{10}$ June 28.</th>
<th>$Q_{10}$ June 30.</th>
<th>$Q_{10}$ July 3.</th>
<th>Average.</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-12</td>
<td>12.72</td>
<td>12.71</td>
<td>12.71</td>
<td>12.71</td>
</tr>
<tr>
<td>3-13</td>
<td>11.2</td>
<td>11.35</td>
<td>11.25</td>
<td>11.23</td>
</tr>
<tr>
<td>4-14</td>
<td>11.7</td>
<td>11.63</td>
<td>11.69</td>
<td>11.67</td>
</tr>
<tr>
<td>5-15</td>
<td>10.80</td>
<td>10.90</td>
<td>10.85</td>
<td>10.85</td>
</tr>
<tr>
<td>6-16</td>
<td>10.00</td>
<td>9.97</td>
<td>10.02</td>
<td>9.99</td>
</tr>
<tr>
<td>7-17</td>
<td>9.67</td>
<td>9.68</td>
<td>9.65</td>
<td>9.66</td>
</tr>
<tr>
<td>8-18</td>
<td>8.52</td>
<td>8.50</td>
<td>8.51</td>
<td>8.51</td>
</tr>
<tr>
<td>9-19</td>
<td>6.95</td>
<td>6.94</td>
<td>6.92</td>
<td>6.93</td>
</tr>
<tr>
<td>10-20</td>
<td>5.62</td>
<td>5.61</td>
<td>5.65</td>
<td>5.63</td>
</tr>
<tr>
<td>11-21</td>
<td>4.72</td>
<td>4.76</td>
<td>4.72</td>
<td>4.73</td>
</tr>
<tr>
<td>12-22</td>
<td>3.90</td>
<td>3.89</td>
<td>3.92</td>
<td>3.90</td>
</tr>
</tbody>
</table>

In Table I the values for the temperature coefficients are given for three quite typical experiments. It will be seen that there is very close agreement between the several values for the same temperature. In the last column are given the averages for these values. Also it will be noted that in all of the experiments with an increase in temperature there is a marked decrease in the values of the coefficients, similar to that found in other physiological processes. That this is not the case in the great majority of ordinary chemical reactions is well known, for in these the temperature is more nearly a constant equal to about 2.5. If we assume that the intensity of the light depends upon the reaction velocity in the luciferin-luciferase system, then the discrepancy in the temperature coefficients must be explained. It is possible, indeed probable, that some factor other than the reaction velocity determines the intensity of the light, and that the observed curve is the resultant of these two reactions.

The use of the critical thermal increments, the $\mu$ of Arrhenius,
for biological reactions in which the velocity of a reaction is a function of the temperature has been discussed at some length by Crozier.

This author is of the opinion that by using the thermal increment rather than the temperature coefficient reactions may be grouped into classes according to the values which they give. In view of this

Fig. 3. Data of Fig. 1 plotted as log brightness against the reciprocal of the absolute temperature.
work it was thought advisable to plot the results in the manner which he suggests. The curve resulting from such a procedure is shown in Fig. 3.

At once one is struck with the similarity between this curve and some which Crozier shows for other biological processes. There is the same intersection of straight lines which he found, the only difference being that here the lines cross at a lower point (about 8°C.) than that found in other reactions. This, however, may be explained on the ground that the optimum temperature for this reaction is at a lower point than that of ordinary biological reactions and one might therefore expect the break to occur at a lower temperature than in the processes analyzed by Crozier. \( \mu \) calculated from the Arrhenius equation

\[
\frac{1}{2} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)
\]

\( K_2 = K_1 e \)

gives a value of 22,440 for the lower curve and 16,590 for the upper curve.

**DISCUSSION.**

What processes actually take place within cells have been the subject of many biological papers, but at this time our knowledge of these processes has barely passed the observational stage, and we are still in the dark as to the sequence of events and the relative importance of the intermediate steps in the formation of an intercellular compound. In a reaction, such as the one considered in this paper, one is justified in using some outstanding and characteristic feature as an index of the reaction velocity of the underlying process. In this case it is the production of light, but to say that when one measures the intensity of the light he is measuring the velocity of the light reaction only, would be erroneous, I believe. Although the production of light by living bacteria is in no way connected with their respiration, it is closely connected with their metabolic processes and ends with their death, so that changes which affect their metabolic activities would affect, of necessity, the production of light. Thus we obtain, I believe, a curve which is not that of the simple light reaction itself, but rather the resultant curve of
several processes which go on simultaneously in the bacterial cell. Indeed this view is substantiated by the form which the curve in Fig. 3 takes. As Crozier has pointed out, this type of curve may be taken as representing a complex reaction, each separate portion of the curve representing control by a separate reaction which is going on and which will influence the rate of the principle activity, that of the production of light.

As a general rule chemical reactions are increased by a rise in temperature in a manner that has been shown by van't Hoff to be equal to two- or threefold for every 10°C interval. If such reactions are put in the form of a curve, they will at first slowly rise and then with increasing steepness rapidly become nearly vertical. However, in physiological problems this effect of heat holds only up to a certain point, which varies according to the conditions; up to this point an increase in temperature accelerates the rate of the reaction, but a further rise slows the reaction down, thus bringing about the state of "optimum temperature."

Bayliss, in his monograph on the nature of enzyme action, holds that this effect of heat brings about a state of coagulation of the colloidal particles in the enzyme. In such a coagulation, if the increase in temperature is not too rapid, there occurs a gradual clumping together of the particles and a lessening of the surface over which they are effective. Thus there would be a tendency for the temperature-velocity curve of a physiological phenomenon to rise less steeply and the van't Hoff rule to be abandoned.

From a comparison with other data cited by Crozier (Crozier, b) one may consider that the reaction, or reactions, which this curve (Fig. 3) represents are mainly oxidative in nature. For other forms this same conclusion has been reached in an entirely different manner (Harvey, e). However, there is apparently another factor which enters into a consideration of the phenomenon, for the values for μ which are obtained point to a process of hydrolysis which goes on at the same time as the process of oxidation. It may be that this process of hydrolysis is accomplished by some enzyme in the bacterial cell which is not directly responsible for the production of light, but which controls some other process linked up very closely with the general metabolism of the cell and which at low temperatures is
slower than that of the luciferin-luciferase reaction, hence acts as a limiting factor in the production of light.

SUMMARY.

1. A method has been described whereby the intensity of the light of luminous bacteria may be measured in a quantitative manner.
2. It is pointed out that the temperature coefficients for light intensity do not follow the van't Hoff rule, but are higher and vary with each 10° temperature interval.
3. From a comparison with other data it is found that the process is not a simple one, but that the observed curve is the resultant of several reactions which proceed simultaneously.
4. The discrepancies in the temperature coefficients in the neighborhood of the "optimum temperature" may be due to a process of coagulation of the colloidal particles of the enzyme. This coagulation will tend to cause a deviation of the curve away from that normal for chemical reactions.

I wish to express my sincere thanks to Professor E. Newton Harvey, of Princeton University, whose interest in the work has made its completion possible. I also wish to express my deep obligation to Dr. W. E. Forsythe, Director of the Nela Research Laboratories, and to the members of his staff, especially Dr. A. G. Worthing and Dr. E. Q. Adams. Dr. Forsythe was kind enough to place a fellowship in the Nela Research Laboratories at my disposal during the summer months of 1924, and it was there that the major part of this work was done.

BIBLIOGRAPHY.

Beijerinck, M. W., Arch. néerl. sc. exactes, 1891, xxiv, 369.
Dubois, R., Compt. rend. Soc. biol., 1887, xxxix, 564.
Forster, J., Centr. Bakt., 1892, xii, 431.


