THE TOTAL LUMINOUS EFFICIENCY OF LUMINOUS BACTERIA.

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INTRODUCTION.

The statement is often made that light production by luminous organisms, such as the fire-fly, is about 95 per cent efficient. This is perfectly true if we refer only to the radiant luminous efficiency, the ratio of the visible radiation (the radiation which we see as light) $\times$ visual sensibility (which takes into account the fact that some colors appear brighter than others, although of equal energy content), to the total radiant energy emitted. As the fire-fly or luminous bacteria emit radiation only in the visible region, with no infra-red or ultra-violet, it is perfectly apparent why the luminous efficiency is so high. But this tells us nothing regarding the radiational efficiency, the efficiency by which energy supplied to a light source is transformed into radiant energy, including all wave-lengths, whether we can see them as light or not. The total efficiency is the product of the two, radiational efficiency $\times$ radiant luminous efficiency, and nobody knows what the total efficiency of any luminous organism is. At best one can only calculate what the total efficiency of animal luminescence might be, on the assumption that the radiational efficiency is about the same as the efficiency of other processes in other animals (Karrer, 1918; Ives, 1922). The radiational efficiency of luminous animals has never been measured and the present paper on luminous bacteria is an attempt to rectify this deficiency. The radiant luminous efficiency of luminous bacteria must be about the same as that of the fire-fly.

Luminous bacteria were selected for the work because they can be grown with great ease in the laboratory and they produce light...
continuously, irrespective of stimulation. Cultivation was made on 2 per cent bacto-peptone nutrient agar (⅓ peptone or protein derivatives) containing 1 per cent (by volume = 1.26 per cent by wt.) of glycerol in sea water, in large enameled pie plates with covers, serving as Petri dishes. The pH was about 7.8. Two strains of bacteria were used; one (Bacterium phosphorescens), isolated by Mr. T. F. Morrison in 1924, and a second (variety of B. phosphorescens) isolated by me in 1925, both from fish at the Princeton Fish Market. I take great pleasure in thanking Mr. Morrison for many samples of his organism which he very kindly grew for me.

Sometimes the light produced on a culture plate would be very bright, at other times not so bright, varying with temperature and unknown factors. One easily learned to tell from the appearance of a pie plate whether the amount of light from a single bacterium would be large or not. This quantity was actually determined in a manner to be described later. Mr. Morrison’s bacterium measured 3.1 × 1.0μ in size, with a volume of 2.239 × 10⁻¹² cc., while my form was 1.1 × 2.2μ, with a volume of 1.695 × 10⁻¹² cc., only .76 as large. Based on the same volume, my bacterium gave off over ten times as much light. For this reason, as well as because my later experiments were more accurate, due to perfection of technique and elimination of unforeseen errors, my final conclusions are based on the work with that form. Twenty-nine experiments in all were carried out, sixteen with the new bacterium.

The experiments were started at the Nela Research Laboratories, Cleveland, Ohio, in July, 1924, and finished at Princeton during the winter. I take great pleasure in expressing my appreciation to Dr. W. E. Forsythe, Director of the Laboratory, for many courtesies and help during my stay in Cleveland, and to Dr. A. G. Worthing and Dr. E. Q. Adams for invaluable advice in connection with the light measurements and mathematical treatment of certain phases of the work.

The general scheme of the investigation is briefly as follows: From metabolism experiments on higher animals it is known that the absorption of a liter of oxygen represents the production of a certain number of calories from oxidation of the foodstuffs. The gm. calories per liter of oxygen vary from 4,485 for protein, 4,686 for fat,
to 5,047 for carbohydrate. This "Method of Indirect Calorimetry" allows us to determine the heat production of an animal with exceptionally good agreement with the values directly determined in a calorimeter. If we may be permitted to apply the method to bacteria, living upon certain proportions of protein and glycerol, we can arrive at a figure for the heat production of the organisms per volume of oxygen consumed. If at the same time that the oxygen consumption is measured, the light emitted is also determined, we have all the data for calculating the efficiency of luminous bacteria as emitters of visible radiation. The figures for calories per liter oxygen absorbed are undoubtedly applicable to any organism, since they represent (except in the case of protein) maximum energy of the foodstuffs. 1 gm. of tallow burned completely to CO₂ and H₂O by a guinea pig produces the same amount of heat, as if burnt in a candle, and no organism could obtain any more heat from a gm. of tallow. If incompletely oxidized the foodstuffs would represent less energy, but this would make the total efficiency of luminescence of the bacteria greater than the figure I have found.

It should be kept in mind that we are now regarding the bacterium as a power plant for light production and its efficiency determined in this way represents a minimum efficiency and should be compared with the over-all efficiency of a power plant generating electric light, i.e. its efficiency as a producer of visible radiation calculated from the energy in the coal used to fire the boilers.

For another reason also the efficiency so measured is a minimum efficiency. It is quite certain that not all the oxygen used by a bacterium is concerned in the process of luminescence; some of it, probably a large part, is used in the respiratory oxidations exhibited by all aerobic bacteria, whether luminous or not. Experiment shows that the respiratory oxidations and light production do not parallel each other. It is possible, by treating luminous bacteria with KCN to reduce the oxidations to \( \frac{1}{20} \) of their former rate, while reducing the luminescence to only \( \frac{1}{4} \) of its previous value. Under these conditions the bacteria would show five times the efficiency that we should calculate for the normal bacteria and it is very likely that
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if we could isolate the particular oxidations responsible for luminescence and measure its oxygen consumption and heat production alone, a still greater efficiency might be observed. Bearing in mind these limitations regarding the value of the efficiency found, we may now turn to the methods of measurement and results.

METHODS.

The measurements involved may be discussed under the following heads:
1. The brightness of a bacterial emulsion of given thickness.
2. Absorption in the emulsion and the light from a single bacterium.
3. Counting the bacteria.
5. Effect of KCN on oxygen consumption and luminescence.

(I) The Brightness of a Bacterial Emulsion of Given Thickness.

It is a simple matter to determine the brightness of a bacterial emulsion in sea water of any given thickness, by comparing it with an opal glass surface illuminated by a light source whose distance can be varied, so that the illumination is known. A small 6 volt auto headlight lamp of 21 c.p. served as the comparison light and could be moved along a photometer bench. It was enclosed in a dark house with a window covered with a Wratten No. 62 green filter in order to match the light of the bacteria, which is decidedly greenish. Observation of the bacterial light with a small spectroscope and rather wide slit showed a broad band with red and blue at the ends. As the slit was narrowed the band becomes smaller and its center is at about $\lambda = .51\mu$. The lamp was run at 5.5 volts and a measurement of the transmission of the No. 62 green filter at this color temperature gave .05 transmission. To calibrate the lamp, a careful measurement of the illumination (without the No. 62 green filter) on the opal glass comparison screen was made with a Macbeth illuminometer with the lamp at a given distance, and the inverse square law applied for other distances. The Macbeth illuminometer read directly in foot candles. To convert to lamberts

1 Kindly made by Dr. Forsythe.
we use the formula: foot candles $\times 1.076 \times .82$ (reflection factor of test plate of illuminometer) = millilamberts. This figure must then be multiplied by .05 to allow for the 5 per cent transmission of the No. 62 green filter, to find the actual illumination on the opal glass.

In order to render the scattering of light as nearly comparable as possible in measurements of different thicknesses of emulsion, the rectangular vessels of glass were mirrored in sides and bottom, the front and back of the vessel being of clear glass. One therefore measured the brightness of a bacterial emulsion of given thickness whose lateral dimensions were practically infinite because of the mirrors.

(2) Absorption in the Emulsion and the Light from a Single Bacterium.

It is obvious that the light coming from any finite thickness of bacterial emulsion is less than it should be because the bacteria absorb and scatter some of the light coming from other bacteria. We should like to know the light emitted from a single bacterium without any absorption and scattering. This can be allowed for in two ways, (1) indirect and (2) direct.

(1) Indirect method: By measuring the brightness of a series of thicknesses of bacterial emulsion in a special mirrored box we can plot a curve showing the relation between brightness as ordinate and thickness of emulsion as abscissa. This curve (Fig. 1) rises steeply at first, then slowly and finally becomes horizontal when the absorption is so great that increasing thickness gives no more light, despite the increasing number of bacteria emitting light. The tangent to this curve at zero thickness represents the light that would be emitted if there were no absorption. It can be drawn in its proper place by plotting $\log (B_{\text{max}} - B_t)$ against thickness of emulsion, $t$, in which $B_{\text{max}} =$ brightness of an infinitely thick emulsion and $B_t =$ brightness of $t$ thickness of emulsion. A straight line is obtained from this plotting, and the thickness that corresponds to $\log B_{\text{max}} - \frac{1}{2.3}$ on the straight line will be the thickness of a clear solution (of identical light production) that would emit the same light as an infinite thickness of the actual solution (Fig. 2). The tangent to the curve representing brightness plotted against thick-
ness can then be drawn, connecting 0 thickness and 0 brightness with the thickness of a clear solution giving $B_{max}$ and the brightness without absorption for a 1 cm. thick layer of luminous bacteria read on the tangent (Fig. 1).

(2) Direct method: The absorption can be measured directly, using the light transmitted by the No. 62 Wratten filter, on the assumption that its spectral distribution agrees fairly closely with that of the bacterial luminescence. In practice two mirrored rec-

![Graph](image)

**Fig. 1.** Relation between the brightness of a bacterial emulsion and its thickness. Experiment of Nov. 18, 1924. Brightness in arbitrary units such that $308 = 0.0399$ millilamberts. The tangent represents the brightness if there were no absorption of light in the emulsion.
inverse square scale set at 10. A match was then obtained between the two fields by varying the current through the illuminometer lamp. When a match is obtained, without changing the positions of the apparatus, the rectangular absorption vessel is filled with the bacterial emulsion, allowed to become dark and non-luminescent through lack of oxygen, and the inverse square scale moved until another match is obtained. This gives the transmission directly,
since the vessel filled with water (scale at 10) = 100 per cent transmission. To find the transmission of a 1 cm. thick layer, $T_1$, we use the following equation.

$$ T_1 = \sqrt[\frac{n}{T_n}] $$

in which $T_n$ is the transmission of a layer of bacterial emulsion, $n$ cm. thick (Table I, Column 4).

If the brightness is measured for a given thickness of rectangular vessel, (Table I, Column 5, for 2.7 cm. thick vessel) we can use the following formula to calculate the brightness of a 1 cm. thick layer of emulsion, if there were no absorption, $B_1$.

$$ B_n = \frac{B_1 (T_n - 1)}{\log T_1} $$

in which $B_n$ = brightness of $n$ thickness. $T_n$ = transmission of $n$ thickness and $T_1$ = transmission of 1 cm. thickness (Table I, Column 6).

The brightness of a 1 cm. thick layer of bacterial emulsion without any absorption, $B_1$, is obtained in millilamberts. A similar surface emitting light would give the same milliunits per sq. cm. And a cube emitting light in all directions would give four times the milliunits per cm$^2$. Consequently it is a simple matter to calculate the lumens per cc. (Table I, Column 7) and, knowing the number of bacteria per cc. (Table I, Column 8) to obtain the lumens per bacterium, (Table I, Column 9). Divided by $4\pi$, we have the candle power of a single bacterium.

In two experiments in which the light emitted by 1 cm. of bacterial emulsion was determined in the two different ways, one gave identical results and the other gave values differing by about 10 per cent. The agreement seemed to be fairly good and as the time necessary to make a measurement became an important factor in the later experiments on oxygen consumption, most of my measurements have been made by determining the absorption directly, a much more rapid method. Where two vessels of different thickness were used the $B_n$ values usually come out within 10 per cent of each other, but the thicker vessel always gives the greater value.
(3) Counting the Bacteria.

An emulsion of luminous bacteria is prepared by gently scraping the bacteria from the nutrient agar surfaces with a camel's hair brush, into sea water. No agar fragments are removed but a very small amount of nutrient material may dissolve. However, the bacteria do not actively grow in the sea water. They may increase at most 15 to 20 per cent in 2 or 3 hours. As the count of bacteria was usually made at the end of the experiment, lasting perhaps 2 hours, the values for light from one bacterium are perhaps a little low.

Measurements show that the light from an emulsion, through which air is always passed in a vigorous stream to prevent the bacteria from using up the supply of oxygen, may increase slightly at first and then diminish, or may remain stationary for a couple of hours, or may slowly diminish in brightness over the period of the experiment. The behavior seems to depend on the age of the cultures, young cultures usually increasing slightly in brightness and old ones dropping off in intensity fairly quickly. The question always arises as to whether all the bacteria one counts are luminous or whether some may have stopped producing light. As the light of bacterial emulsions usually falls off slowly with time, some bacteria must cease to luminesce or all the bacteria must diminish in intensity. In either case the amount of light from a single bacterium would be lower than it should be and I therefore feel justified in selecting the highest values obtained as probably representing reality.

The counts of bacteria were made, after one-fifth dilution, on a blood counting slide. A trace of CuSO₄ was added to kill the moving bacteria and time allowed for the bacteria to settle to the ruled squares on the slide in a moist chamber. Different counts of the same emulsion were in surprising agreement.

With Mr. Morrison's strain of bacteria, counting on a slide was impossible because of the tendency to clump. Accordingly, careful camera lucida drawings of the bacteria under oil immersion objectives, were made by Mrs. Harvey, the organisms measured, and the average volume calculated. Then about 200 cc. of bacterial emulsion was centrifuged to constant volume of bacteria and the
number determined by dividing this volume by the volume of a single bacterium. In two experiments a count was made by both methods. The results were:

<table>
<thead>
<tr>
<th>Method</th>
<th>1st experiment.</th>
<th>2nd experiment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>By centrifugal method</td>
<td>$25.55 \times 10^8$ per cc.</td>
<td>$37.3 \times 10^8$ per cc.</td>
</tr>
<tr>
<td>&quot; counting on slide.</td>
<td>$27.20 \times 10^8$ &quot; &quot;</td>
<td>$36.0 \times 10^8$ &quot; &quot;</td>
</tr>
</tbody>
</table>

The results agree very well. Nevertheless I believe the greatest source of error in these experiments is introduced in counting the bacteria. This is because the bacteria were usually counted at the end of an experiment when they had increased somewhat, perhaps 20 per cent. As previously stated this will make the values for light from a single bacterium too low. The values for efficiency are calculated on the basis of light emitted per mg. of oxygen consumed, for the same number of bacteria in each case. However, any non-luminescing bacteria in the emulsion will reduce the light per mg. of oxygen consumed because of their absorption. They also utilize oxygen. I therefore feel justified in selecting the highest values for light per mg. of oxygen absorbed in calculating the efficiency of the organisms.

(4) Measuring Oxygen Consumption.

My first attempts to measure oxygen consumption by the bacteria were made with the Haldane-Henderson gas analysis apparatus. The bacterial emulsion had to be brought into equilibrium with a gas phase, a slow process. In addition, my respiratory quotients ($\frac{CO_2}{O_2}$) did not give constant values so that I abandoned this method and turned to a simpler one, which measures only oxygen consumption, but which can be carried out very quickly and probably gives values sufficiently accurate for the purpose. de Coulon (1916) has used it in studying oxygen consumption under the influence of anesthetics and other substances.

If we shake an emulsion of luminous bacteria in sea water with
air and then allow it to stand undisturbed in a tall narrow tube without temperature changes, the bacteria will use up all the oxygen and the emulsion become dark. The time for darkening is inversely proportional to the number of bacteria present, provided the darkening does not occur too rapidly. Once the darkening begins, the luminescence fades very quickly until the tube is completely dark except at the surface in contact with the air. My measurements show that a tube may stand for 15 minutes without any diminution in light intensity, when the luminescence will suddenly begin to diminish and the tube is completely dark in 1 or 2 minutes more.

Morrison\(^3\) has determined that the luminescence of luminous bacteria remains constant as the oxygen concentration is diminished until about 2 per cent oxygen is present in the gas in equilibrium with the bacterial emulsions. Therefore if we determine the time necessary for a given emulsion of bacteria to use up its own oxygen, from saturation at atmospheric pressure (say 20 per cent oxygen) to the point where the light just begins to fade (2 per cent oxygen), we can get the average time for utilization of 90 per cent of the dissolved oxygen (Table I, Column 10).

Fox (1909) has made careful determinations of the amount of oxygen dissolved by 1 cc. sea water from a free dry atmosphere of 760 mm. air at various temperatures. Correcting this value for water vapor and multiplying by .9, gives us the volume of oxygen used by the bacteria in 1 cc. of emulsion\(^4\) in a given time.

The experiment is actually carried out by making a series of oxygen consumption determinations and also a series of brightness measurements at various times over a period of 2 hours. The oxygen consumption and brightness as ordinates are then plotted against time as abscissæ and curves drawn for the two values (see Fig. 3).

Both light intensity and oxygen consumption usually diminish with time, but they do not parallel each other. The oxygen consumption reaches a fairly constant value in the course of 45 minutes while the luminescence continues to diminish. From the curves of Fig. 3 one can see that a maximum light for the oxygen consumed

\(^3\) Experiments to be published shortly.

\(^4\) These values are given in Column 11 of Table 1 for the temperatures recorded in Column 2.
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will be given at a certain time and this has been selected in calculating the efficiency. The light value used is recorded in Column 16 of Table I.

Some organisms consume oxygen at a rate proportional to the oxygen concentration, while others use equal amounts of oxygen in a given time, independently of the oxygen concentration (see Amber-

![Graph showing brightness and oxygen consumption over time](image)

**Fig. 3.** Brightness of a bacterial emulsion in arbitrary units (46.3 = .0333 millilamberts) and oxygen consumption (time in minutes to use up a given amount of oxygen) plotted against time. Note that the upper and lower curves are plotted for different ordinates. Experiment of Mar. 11, 1925.

son, 1924–25) I have not yet studied luminous bacteria carefully enough to make definite statements for the whole range of oxygen concentration, but I have studied the time necessary for emulsions of luminous bacteria to darken when the oxygen concentration is 20 per cent (air) and 100 per cent (pure oxygen); and 20 per cent (air) and 4 per cent ($\frac{1}{5}$ air, $\frac{4}{5}$ hydrogen). If the bacteria use equal
amounts of oxygen in equal times (straight line relation) we should expect that when the oxygen concentration \( c \) is multiplied by 5, the time to darken would be slightly greater than 5 times that for \( c \) to darken; whereas if the bacteria use oxygen in proportion to its concentration (logarithmic relation) we should expect that when the oxygen concentration \( c \) is multiplied by 5, the time to darken will be much less than 5 times that for \( c \) to darken.

My figures (averages of four to six determinations) are as follows:

<table>
<thead>
<tr>
<th>Conc. of oxygen</th>
<th>Time to darken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure oxygen (100 per cent ( \text{O}_2 ))</td>
<td>1,441 sec</td>
</tr>
<tr>
<td>Air (20 per cent ( \text{O}_2 ))</td>
<td>249 sec</td>
</tr>
<tr>
<td>&quot; (20 &quot; &quot; ( \text{O}_2 ))</td>
<td>870 sec</td>
</tr>
<tr>
<td>&quot; 1 part, hydrogen 4 parts (4 per cent ( \text{O}_2 ))</td>
<td>166 sec</td>
</tr>
</tbody>
</table>

It will be observed that 5\( c \) concentration takes about 5 times as long as \( c \) concentration and a straight line relation must hold. The luminescence intensity is about the same in all these oxygen concentrations (becomes less intense in pure oxygen). Below 2 per cent oxygen the luminescence intensity diminishes very quickly with decrease in oxygen concentration, and one experiment indicates that with 3.6 per cent oxygen the time to darken was 300 seconds while with half that amount (1.8 per cent oxygen) 260 seconds was required to darken. These figures do not agree with a straight line relation and I presume that below 2 or 3 per cent oxygen a logarithmic relation may hold.

In measuring the oxygen consumption of an emulsion I always take the time to the point where the tube just begins to darken (about 2 per cent oxygen) and I feel that this procedure must give a fairly accurate value (Table I, Column 10). The efficiency of luminescence is calculated from oxygen consumption as follows: The burning of a gm. molecular weight of glycerol liberates 397,200 gm. calories and involves the absorption of 112 gm. or 78.3 liters of oxygen. Hence 1 liter of oxygen burning glycerol is equivalent to 5,070 gm. calories.

1 liter of oxygen burning peptone is equivalent to 4,485 gm. cal-
## Table I. Results of Experiments on Oxygen Consumption and Lumens Emitted by One Luminous Bacterium.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Temperature °C</th>
<th>Transmission of 0.1 cm. of black vessel</th>
<th>Transmission of 1 cm. of black vessel</th>
<th>Brightness in millibereau of 0.1 cm.</th>
<th>B, lumen per cc.</th>
<th>Brightness in millibereau of 1 cm.</th>
<th>B × 100</th>
<th>Lumen emitted per bacterium X 100</th>
<th>Oxygen available for utilization per cc.</th>
<th>Oxygen absorbed per sec. by 1 cc. bacterial emission X 100</th>
<th>Oxygen used by 1 bacterium per sec. X 100</th>
<th>Oxygen used by 1 bacterium on basis of figures of Column 12 X 100</th>
<th>Lumens per mg. of oxygen used to calculate lumens per mg. oxygen</th>
<th>Remarks: bacterial content at beginning of experiment denoted by E, at end by E, bacteria counted at end by E.E. 25.5 bacteria per cc. by centrifuge method. E. Bacteria counted in middle of experiment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 2</td>
<td>21</td>
<td>55.0</td>
<td>80.9</td>
<td>.0561</td>
<td>.0265</td>
<td>105.8</td>
<td>11.07</td>
<td>9.57</td>
<td>6.50</td>
<td>7.23</td>
<td>6.52</td>
<td>9.35</td>
<td>10.23</td>
<td>9.57</td>
</tr>
<tr>
<td>&quot;</td>
<td>18</td>
<td>49.4</td>
<td>76.9</td>
<td>.0237</td>
<td>.0123</td>
<td>49.0</td>
<td>11.23</td>
<td>4.36</td>
<td>9.80</td>
<td>5.22</td>
<td>4.65</td>
<td>6.65</td>
<td>6.56</td>
<td>4.36</td>
</tr>
<tr>
<td>&quot;</td>
<td>10</td>
<td>29.0</td>
<td>63.5</td>
<td>.0704</td>
<td>.0450</td>
<td>180.2</td>
<td>27.20</td>
<td>6.63</td>
<td>23.0</td>
<td>19.42</td>
<td>19.60</td>
<td>10.10</td>
<td>5.44</td>
<td>5.50</td>
</tr>
<tr>
<td>&quot;</td>
<td>11</td>
<td>43.6</td>
<td>73.6</td>
<td>.1445</td>
<td>.0785</td>
<td>314.0</td>
<td>13.46</td>
<td>23.30</td>
<td>408</td>
<td>4.42</td>
<td>10.70</td>
<td>7.95</td>
<td>11.37</td>
<td>14.07</td>
</tr>
<tr>
<td>&quot;</td>
<td>17</td>
<td>43.2</td>
<td>73.1</td>
<td>.0468</td>
<td>.0257</td>
<td>102.9</td>
<td>8.40</td>
<td>12.14</td>
<td>4.32</td>
<td>10.46</td>
<td>12.36</td>
<td>17.67</td>
<td>6.30</td>
<td>11.12</td>
</tr>
<tr>
<td>&quot;</td>
<td>18</td>
<td>38.5</td>
<td>70.3</td>
<td>.1360</td>
<td>.0780</td>
<td>312.0</td>
<td>13.00</td>
<td>24.00</td>
<td>372</td>
<td>4.54</td>
<td>12.20</td>
<td>9.39</td>
<td>13.43</td>
<td>13.72</td>
</tr>
<tr>
<td>Apr. 8</td>
<td>22</td>
<td>29.4</td>
<td>64.6</td>
<td>.1040</td>
<td>.0654</td>
<td>261.5</td>
<td>20.60</td>
<td>12.70</td>
<td>222</td>
<td>4.54</td>
<td>20.5</td>
<td>10.00</td>
<td>14.30</td>
<td>8.46</td>
</tr>
</tbody>
</table>
ories. As the bacteria fed upon 0.8 gm. peptone or protein derivatives for each 1.26 gm. glycerol (about 40 per cent peptone and 60 per cent glycerol), the expected gm. calories per liter oxygen absorbed will be 4,840 on this proportional diet. Even if this value is not strictly correct the true value could not differ from this by more than 7 per cent in either direction.

Knowing the lumens produced per oxygen absorbed per second (Table I, Column 15) and the calories represented by 1 liter of oxygen, we have the light produced per calorie input per second. Multiplied by 4.184, calories become watt-seconds, and the lumens per watt may be stated. As 640 lumens per watt represents the maximum possible efficiency, it is an easy matter to express in percentage, the efficiency of the normal luminous bacteria as light emitters.

(5) Effect of KCN on Oxygen Absorption.

Studies on oxygen consumption and luminescence intensity show that in general, rapidly respiring cells are also brightly luminescing cells but the relation is by no means quantitative as my values for the lumens per mg. of oxygen consumed show. The variations are too great to be accounted for by experimental errors, especially as a count of bacteria does not enter into the calculations. It is well known that KCN inhibits the respiratory oxidations of practically all cells, and I have observed (1917, a, b) that the luminescence of many forms is not affected by KCN. The brightness of bacterial luminescence is reduced somewhat, depending on the concentration of KCN, while the absorption of oxygen is reduced a great deal. As the result of a large number of experiments in which the brightness and oxygen consumption of bacterial emulsions containing KCN was compared with the brightness and oxygen consumption of control bacterial emulsions without KCN over the same time period, I have come to the conclusion that in \( \frac{N}{5,000} \) KCN, oxygen consumption may be reduced to .05 of its normal value while brightness is reduced only to .25 of its normal value. Thus we should have in \( \frac{N}{5,000} \) KCN, five times as much light produced for a given oxygen
consumption. In these experiments the absolute amount of light produced was not determined but the brightness of a given thickness (3 cm.) of emulsion with KCN was compared at the same time with the brightness of the same thickness of control emulsion, regarded as 100 per cent.

By the use of KCN we can partially separate luminescence oxidations from respiratory oxidations and one is justified, I believe, in applying this correction to the efficiency. By ruling out some of the energy-yielding oxidations which are not concerned in the production of light we obtain a fivefold increase in luminescence efficiency. One would like to separate completely the luminescence from the other processes in the cells. Perhaps a substance may be found that will inhibit completely the respiratory oxidations while leaving the luminescence unchanged. Then a true value for luminescence efficiency could be given.

RESULTS.

In Table I are recorded the values obtained in seven experiments performed after my technique was perfected, and which give representative results.

It will be observed (Column 15) that the maximum recorded lumen-seconds per mg. of oxygen consumed is 14.07. The average of the seven experiments is 9.25. Selecting the maximum on the ground, that, in the other experiments, some bacteria may be respiring but not luminescing, we calculate the efficiency as follows: On the bacterial diet used, 1 liter of oxygen absorbed will give 4,840 gm. calories from oxidation, or 1 cc. oxygen absorbed will give 4.84 gm. calories. 1 cc. O_2 weighs 1.43 mg. so that \( \frac{4.84}{1.43} \) = 3.38 calories per mg. Since 3.38 \( \times 4.184 \) = 14.1 watt-seconds per mg. O_2 absorbed and 14 lumen-seconds per mg. O_2 are emitted, about 1 lumen per watt is realized or \( \frac{1}{640} \) = .00156 efficiency for the normal bacterium carrying out other processes besides light production. Taking into account the partial separation of luminescence and respiratory oxidations effected by KCN, we can multiply the efficiency by 5 and obtain 5 lumens per watt or .0078 efficiency.
In comparing this value with the efficiencies of present day illuminants (see Ives, 1915 and Karrer, 1915) I think it is only proper to estimate the efficiency of the commercial illuminant from the heat value of the illuminating gas or the heat value of the fuel used to generate current for electric light, since we use the heat value of the fuel (food) of the bacteria in estimating their efficiency. On this basis, the bacterial efficiency is well above that of any gas illuminants. Accepting 20 per cent as an average over-all efficiency for large modern steam-power plants generating electric current,\(^5\) the 8 lumens per watt of a tungsten vacuum and the 19.6 lumens per watt of a tungsten nitrogen lamp become 1.6 and 3.92 lumens per watt, respectively. Thus the bacteria are somewhat ahead of our modern illuminants. While the extravagant claims of efficiency made for luminous animals are not borne out by this investigation, the values are sufficiently high to warrant further inquiry into the process of light production, especially in other forms which produce a much brighter light than the bacteria. One would like to know the efficiency of the light-producing process of the bacteria alone, when completely separated from the other energy-consuming processes of the organism.

A few other calculations are of interest. The maximum observed candle power of a single bacterium (length 2.2\(\mu\); diameter 1.1\(\mu\)), whose volume is 1.695 \(\times\) 10\(^{-12}\) cc. is 24 \(\times\) 10\(^{-14}\) lumens divided by 4\(\pi\) or 1.9 \(\times\) 10\(^{-14}\) candles. Assuming the bacteria to be cylinders 1.1\(\mu\) long and 1.1\(\mu\) in diameter, with a hemisphere at each end of .55\(\mu\) radius, the area of a single bacterium will be 7.6\(\mu^2\) or 7.6 \(\times\) 10\(^{-8}\) sq. cm. Hence the specific luminous emission or intrinsic brilliancy is
\[
\frac{24 \times 10^{-14} \text{ lumens}}{7.6 \times 10^{-8} \text{ cm}^2} = 3.14 \times 10^{-6} \text{ lumens per cm}^2.
\]

Since a liter of oxygen at N. T. P. contains 2.7 \(\times\) 10\(^{23}\) molecules and the bacteria (Mar. 11 exp.) use 7.95 \(\times\) 10\(^{-18}\) liters of oxygen per second, 215 \(\times\) 10\(^8\) molecules of oxygen are absorbed per second. The same bacteria absorbing oxygen at the above rate emitted 16 \(\times\) 10\(^{-14}\) lumens per second. At the wave-length of maximum energy of bacterial light (510\(\mu\mu\)) 323 lumens are equivalent to 1 watt. Hence

\(^5\)Figures kindly supplied for me by Dean A. M. Green, Jr., of the Engineering School of Princeton University.
the bacteria emit \( \frac{16 \times 10^{-14}}{323} = 4.95 \times 10^{-16} \) watts or \( (\times 10^7) 4.95 \times 10^{-9} \) ergs per second. As one quantum of light at \( \lambda = 510 \mu \text{m} \) is equivalent to \( 3.86 \times 10^{-12} \) ergs, we see that \( \frac{4.95 \times 10^{-9}}{3.86 \times 10^{-12}} = 1,280 \) quanta of light per second are produced per bacterium.

Now suppose that a molecule of oxygen uniting with the luminous material results in the emission of 1 quantum of light energy. We would have \( 215 \times 10^3 \) molecules \( \text{O}_2 \) absorbed, and \( 1.28 \times 10^3 \) molecules \( \text{O}_2 \) used in luminescence, or about \( \frac{1}{168} = .00596 \) of the oxygen absorbed is used in luminescence. When we calculate efficiency on the assumption that all the oxygen absorbed is used in luminescence, we obtain 1 lumen per watt. If only \( \frac{1}{168} \) is utilized in luminescence we should have 168 lumens per watt produced by the bacteria, instead of 1 lumen per watt, and our efficiency would be \( \frac{168}{640} = 26.2 \) per cent.

The above calculation is no doubt an interesting one, but further work is necessary to justify the validity of our assumptions and I would not put too much reliance upon this value.

**SUMMARY.**

Methods are described for measuring the light emitted by an emulsion of luminous bacteria of given thickness, and calculating the light emitted by a single bacterium, measuring \( 1.1 \times 2.2 \) micra, provided there is no absorption of light in the emulsion.

At the same time, the oxygen consumed by a single bacterium was measured by recording the time for the bacteria to use up .9 of the oxygen dissolved in sea water from air (20 per cent oxygen). The luminescence intensity does not diminish until the oxygen concentration falls below 2 per cent, when the luminescence diminishes rapidly. Above 2 per cent oxygen (when the oxygen dissolving in sea water from pure oxygen at 760 mm. Hg pressure = 100 per cent) the bacteria use equal amounts of oxygen in equal times, while
below 2 per cent oxygen it seems very likely that rate of oxygen absorption is proportional to oxygen concentration. By measuring the time for a tube of luminous bacteria of known concentration saturated with air (20 per cent oxygen) to begin to darken (2 per cent oxygen) we can calculate the oxygen absorbed by one bacterium per second.

The bacteria per cc. are counted on a blood counting slide or by a centrifugal method, after measuring the volume of a single bacterium (1.695 × 10⁻¹² cc.). Both methods gave results in good agreement with each other.

The maximum value for the light from a single bacterium was 24 × 10⁻¹⁴ lumens or 1.9 × 10⁻¹⁴ candles. The maximum value for lumen-seconds per mg. of oxygen absorbed was 14. The average value for lumen-seconds per mg. O₂ was 9.25. The maximum values were selected in calculating the efficiency of light production, since some of the bacteria counted may not be producing light, although they may still be using oxygen.

The “diet” of the bacteria was 60 per cent glycerol and 40 per cent peptone. To oxidize this mixture each mg. of oxygen would yield 3.38 gm. calories or 14.1 watts per second. 1 lumen per watt is therefore produced by a normal bacterium which emits 14 lumen-seconds per mg. O₂ absorbed. Since the maximum lumens per watt are 640, representing 100 per cent efficiency, the total luminous efficiency if .00156.

As some of the oxygen is used in respiratory oxidation which may have nothing to do with luminescence, the luminescence efficiency must be higher than 1 lumen per watt. Experiments with KCN show that this substance may reduce the oxygen consumption to $\frac{1}{20}$ of its former value while reducing the luminescence intensity only $\frac{1}{4}$.

A partial separation of respiratory from luminescence oxidations is therefore effected by KCN, and our efficiency becomes 5 lumens per watt, or .0078. This is an over-all efficiency, based on the energy value of the “fuel” of the bacteria, regarded as a power plant for producing light. It compares very favorably with the 1.6 lumens per watt of a tungsten vacuum lamp or the 3.9 lumens per watt of a
tungsten nitrogen lamp, if we correct the usual values for these illuminants, based on watts at the lamp terminals, for a 20 per cent efficiency of the power plant converting the energy of coal fuel into electric current.

The specific luminous emission of the bacteria is $3.14 \times 10^{-4}$ lumens per cm$^2$.

One bacterium absorbs 215,000 molecules of oxygen per second and emits 1,280 quanta of light at $\lambda_{mo} = 510\mu\mu$. If we suppose that a molecule of oxygen uniting with luminous material gives rise to the emission of 1 quantum of light energy, only $\frac{1}{168}$ of the oxygen absorbed is used in luminescence. On this basis the efficiency becomes 168 lumens per watt or 26.2 per cent.

BIBLIOGRAPHY.


Karrer, E., 1918, *J. Franklin Inst.*, clxxv, 775.