

# THE RESPIRATION OF LUMINOUS BACTERIA AND THE EFFECT OF OXYGEN TENSION UPON OXYGEN CONSUMPTION

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## INTRODUCTION

In the case of large organisms the rate of respiratory exchange will depend (1) on the amount of oxygen available for use, and (2) on adequate respiratory and circulatory mechanisms in the body. Amber-son and his associates<sup>3</sup> in the case of several marine invertebrates, and S. Nomura<sup>22</sup> with the Holothurian *Caudina*, have shown that the rate of oxygen consumption is proportional to the oxygen tension of the medium. Pütter<sup>26a</sup> examined the oxygen consumption of several invertebrates at low pressures of oxygen and determined the limiting value for oxygen at which respiratory activity was equivalent to that in air. Very recently Hall<sup>10</sup> has shown the dependence of certain marine fishes upon the amount of available oxygen in the medium and the haemoglobin content of the blood for complete respiration. In all of these larger forms the rate of oxygen consumption is dependent upon oxygen concentration over a wide range of oxygen pressures.

In general, organisms such as protozoa or bacteria are small enough to allow complete diffusion of gases dissolved in the medium, and consume oxygen at a constant rate independently of the oxygen pressure until the oxygen concentration falls to a point allowing incomplete activity of the respiratory mechanism within the cell. Only then does the rate of oxygen consumption diminish, and in many forms this limiting value for adequate respiration will be very small indeed, as has been shown by various workers, (Warburg,<sup>34a</sup> Henze,<sup>13</sup> Lund,<sup>19</sup> Pütter,<sup>26b</sup> Harvey<sup>11b</sup>) whose experiments indicate that in unicellular

organisms oxygen consumption remains independent of oxygen concentration over a very wide range of oxygen pressure. Amberson<sup>2</sup> has shown that the oxygen uptake of *Paramecium* is practically constant from 200 to 50 mm. Hg oxygen, and the limiting value for adequate respiration in these organisms is not reached until the oxygen tension falls below 50 mm. At 11 mm. of oxygen the reduction in the rate of respiration is only 20 per cent of that at atmospheric pressure. Lund<sup>19</sup> has obtained results in agreement with those of Amberson on the same organism. Amberson also conducted experiments on the dividing egg of the sea-urchin and found that respiratory activity does not fall away from the rate at atmospheric pressure of oxygen until 80 mm. Hg is reached, and no marked decrease in the rate of respiration occurs until oxygen pressure has been reduced to 20 mm. Hg.

Estimations have been made of the rate of oxygen consumption with abundant oxygen by luminous bacteria when suspended in seawater, by methods involving the time for dimming (Harvey<sup>11b</sup>) and by a manometric method (Harvey<sup>11c</sup>). In the present investigations measurements have been made of actual values for oxygen consumption and carbon dioxide production when the amount of available oxygen is diminished, and also the rate of respiratory exchange before and following the dimming of luminescence of luminous bacteria due to lack of oxygen. The amount of oxygen necessary for the maximum luminescence is less than that required for maximum metabolism of the cell, and luminescence will still occur when only a very small amount of oxygen is present (Beijerinck,<sup>5</sup> Harvey<sup>11d</sup>). The actual amount of oxygen necessary to give just visible luminescence has been estimated by Harvey and Morrison<sup>11e</sup> to be of the small value 0.0053 mm. Hg (0.0007 per cent). At least 90 per cent of available oxygen is consumed when dimming of a suspension of luminous bacteria occurs.

In these experiments oxygen consumption by luminous bacteria has been followed by two methods: (1) Colorimetric, involving the use of haemocyanin as an indicator of the presence of dissolved oxygen in suspensions of bacteria, and (2) a manometric method allowing direct volumetric determinations to be made of the amount of oxygen consumed in given time.

*Colorimetric Determinations of Oxygen Consumption*

It is necessary to have a convenient indicator for the presence of oxygen if the rate at which oxygen is removed from a suspension of bacteria is to be observed. No colorimetric indicator of oxygen is available in which living cells may be placed excepting the blood-serum of certain crustaceans containing the copper-protein compound haemocyanin. The methods followed in these experiments are similar to those suggested by Osterhout<sup>23</sup> and Harvey<sup>1a</sup> for the use of *Limulus* haemocyanin as an oxygen indicator. Luminous bacteria live perfectly well in the oxygenated *Limulus* serum, and as they consume oxygen from the serum in which they are suspended it passes from the dark blue of fully-oxygenated oxyhaemocyanin to the colorless reduced haemocyanin free of oxygen.

TABLE I  
*Color Standards*

Tube No.	Oxygenated haemocyanin
1	1 part to 9 parts sea-water
2	2 parts " 8 " "
3	3 " " 7 " "
4	4 " " 6 " "
5	5 " " 5 " "
6	6 " " 4 " "
7	7 " " 3 " "
8	8 " " 2 " "
9	9 " " 1 part sea-water
10	10 " " 0 " "

Each tube with 1 cc. bacterial suspension killed with toluol

The color change of a standing tube of *Limulus* serum containing luminous bacteria was followed, and the actual time for the consumption of available oxygen was determined. The experimental tube containing living luminous bacteria in blood-serum was compared with a set of standards from dilutions of oxygenated *Limulus* haemocyanin to represent the shade of blue color present at definite percentages of oxyhaemocyanin. Corresponding oxygen tensions for each percentage of oxyhaemocyanin were taken from the haemocyanin-oxyhaemocyanin dissociation curve of Redfield, Coolidge, and Hurd.<sup>27</sup> Color standards were made as shown in Table I, using ten uniform test-tubes.

The killed bacterial suspension was added to give to the color standards the same degree of turbidity that is exhibited by an experimental tube prepared according to the same method.

A series of indicator tubes prepared as above served for comparison with a standing tube of *Limulus* oxyhaemocyanin containing 1 cc. of *living* luminous bacteria whose oxygen consumption was to be measured. The exact time was

taken whenever the experimental tube compared exactly in color with one of the standards. The blood-serum was thoroughly shaken with air until completely in equilibrium; the time was taken from the moment the luminous bacteria were added to the tube of serum. As the oxyhaemocyanin was reduced by the bacteria in the experimental tube confusing yellowish pigments appeared with the diminution of the blue color of the oxyhaemocyanin. A small amount of Orange G added to the color standards compensated for this color change in the experimental tube.

In Table II the corresponding oxygen tensions and volume percentages of oxygen for each standard tube are given. It will be seen that no color change occurs until the bacteria have reduced the oxygen concentration in the experimental tube to a value corresponding to 28.50 mm. Hg tension (3.75 per cent oxygen). This will correspond with standard Tube 8, one being scarcely able to distinguish a color change between standard No. 8 and standard No. 9, while no visible change from blue to less blue occurs above this value of oxygen. Oxyhaemocyanin is within 10 per cent of complete equilibrium with the air at this value of oxygen, and no distinction in color can be seen between 90 per cent and 100 per cent oxyhaemocyanin.

Considerable time was therefore required for the bacteria to consume enough oxygen to cause a reduction of the oxyhaemocyanin to a point resulting in color changes at which determinations could begin. From a beginning value of 23.50 mm. oxygen to a point equivalent to standard No. 1, or 3.75 mm. (0.493 per cent oxygen), it was possible to follow the changes in the color of the experimental tube and to obtain a curve for color values against time. The oxygen consumption curve obtained by this method is limited only by the extent of the color changes of haemocyanin when passing from the oxygenated to the reduced condition. Fig. 1 shows this relation plotted against time.

At the point of lowest possible concentration of oxygen estimated by the colorimetric method, the suspension of luminous bacteria in the serum was found to be still aglow. Dimming does not occur until after a complete reduction of the oxyhaemocyanin has been brought about. This may be determined by observing the experimental tube in the dark beside a control tube kept shaken and in equilibrium with the air. When the dimming of the experimental tube begins, it is easily noted by comparison with the brilliancy of glow in the control.

Table II gives complete data from a single experiment of oxygen consumption determination.

In this experiment no dimming of the suspension of luminous bacteria was detected until a total time of 16 minutes had elapsed, a point well beyond the time at which visible reduction of the oxyhaemocyanin had occurred. Hence, it is not possible to follow the rate of oxygen consumption beyond the point of dimming by this method. The character of the oxygen consumption curve beyond this point has been investigated by the manometric method to be described later in the present paper.

TABLE II  
*Experiment 2, July 11, 1927*

Time in minutes and seconds	Standard tube No.	Per cent OxyHcy.	Oxygen tension in mm.	Per cent oxygen in suspension	Log. of oxygen tensions
0.00	10	100	—	—	—
1.00	10	100 (?)	—	—	—
2.00	9	90	34.50	4.53	1.538
2.45	8	80	28.50	3.75	1.455
3.10	7	70	23.25	3.27	1.366
3.40	6	60	18.25	2.40	1.264
4.25	5	50	13.75	1.81	1.138
5.30	4	40	10.50	1.38	1.021
6.15	3	30	7.60	1.00	0.881
7.30	2	20	5.25	0.690	0.760
10.00	1	10	3.75	0.493	0.574

It may be noted in Fig. 1 that as oxygen consumption proceeds, equal parts of oxygen are consumed in equal times, independently of oxygen concentration (a "zero-order" reaction), down to a very low value of approximately 20 mm. Hg, before a marked reduction in the rate of oxygen consumption occurs. This is below a point corresponding with standard Tube 7, equivalent to 23.25 mm. oxygen, and standard Tube 6, which corresponds to 18.25 mm. oxygen. This is quite unlike the determinations made for larger animals where the oxygen consumption is proportional to the pressure over long periods of time and wide ranges of pressures. We cannot conclude that oxygen consumption of luminous bacteria is proportional to the oxygen

concentration except possibly at very low values of oxygen below the point of dimming. This is observed also in the complete curve obtained by manometric methods. Dimming of the suspension of bacteria occurs after the decrease in respiratory rate, indicating that the point of dimming is not the exact point at which oxygen concentration becomes just inadequate for maximum respiration of the cell.

The decrease in the rate of oxygen consumption must occur at a point where the active reactant oxygen is diminished to a value not

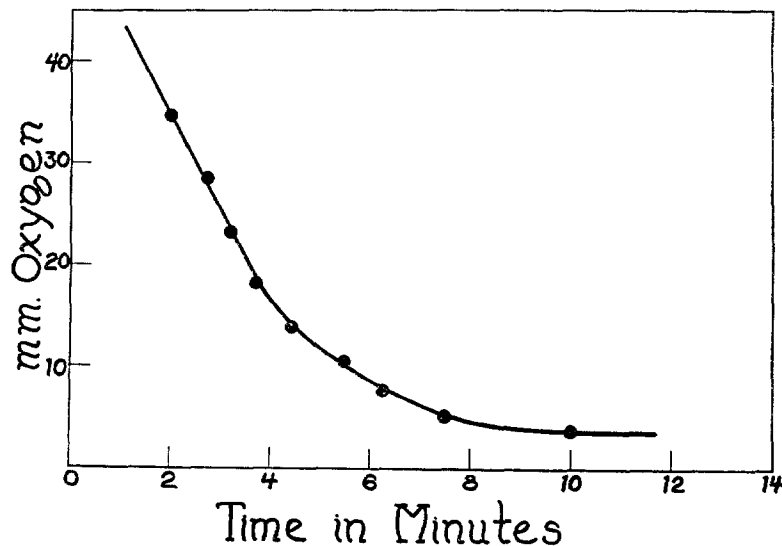


FIG. 1. Oxygen consumption of a suspension of luminous bacteria

quite sufficient to completely activate the respiratory mechanism of the cell, assuming that oxygen diffuses readily into these small organisms. This method for the determination of oxygen consumption is added to these studies to check later observations, and to call attention to the use of a colorimetric method for detection of the rate of oxygen consumption by small organisms. It should be borne in mind that there is no removal of the reaction product, carbon dioxide, but since *Limulus* blood is very well buffered, no inhibitory effects on the activity of the bacteria occurred, and the hydrogen-ion concentration of the serum did not change during the course of an experiment. The

greatest disadvantage of this method is that the range of the indicator is extremely limited and judgment of color values may often be inaccurate.

*Colorimetric Determination of Carbon Dioxide Production by Luminous Bacteria*

The amount of carbon dioxide produced in a standing tube containing a suspension of luminous bacteria in sea-water has also been determined by a colorimetric method. Haas<sup>9</sup> and Saunders<sup>28</sup> have previously used indicator dyes for the determination of carbon dioxide production by both marine and fresh-water animals. Henderson and Cohn<sup>12</sup> have published a table for the relation of the pH of sea-water to its carbon dioxide tension. By the use of indicator dyes it is possible to follow changes in the pH of a bacterial suspension in sea-water as the bacteria produce carbon dioxide and effect the bicarbonate buffer equilibrium.

Allowance was made for the salt-error introduced, from the tables of Kolthoff and Furman,<sup>16</sup> and careful check was made on the changing pH of the suspension in time. Curves plotted for the pH were expressed in terms of carbon dioxide tension according to the data of Henderson and Cohn. Suspensions of the bacteria were always shaken into complete equilibrium with the air and added to a test-tube containing the proper amount of the indicator (Phenol red), while a like amount of the suspension was added to a test-tube without dye to serve as a control for observation in the dark to establish time for dimming. At the end of each observation, when the experimental tube had reached the lowest pH measurable by the indicator, air was bubbled through the suspension to drive off the carbon dioxide produced and allow the suspension to return to near its original pH. This indicated that the change in pH was almost entirely due to the presence of the carbon dioxide produced by the bacteria. If the indicator did not return to within 0.15 of the original pH, the experiment was disregarded.

Although luminous bacteria are very active producers of acid, as Hill<sup>14</sup> has shown, a great acid production occurs only when they are suspended in a medium containing carbohydrates. Since these experiments were always conducted with bacteria suspended in a non-nutrient medium free of carbohydrates, the acid production was very slight, the only source of acid being from the carbohydrates of the cells themselves and dead bacteria in the suspensions. The experiments were conducted over relatively short time intervals, and acid production at no time became too great for reading the pH values or such as to obscure results. It will be seen from Fig. 2 that in a series of experiments on carbon dioxide production in samples from a single suspension of luminous bacteria, there is a slight

rise in the hydrogen-ion concentration of the suspension at all times. This is the only evidence of the slight amount of non-volatile acid that is produced by the bacteria as the suspension becomes older. However, only in old suspensions and suspensions made from old cultures were there sufficient quantities of acid to cause error to determinations. Consequently, all of the experiments were conducted with fresh and young, brilliantly-glowing cultures.

In Fig. 2 the rise in the amount of carbon dioxide produced in the suspension proceeds at a constant rate from equilibrium with the air

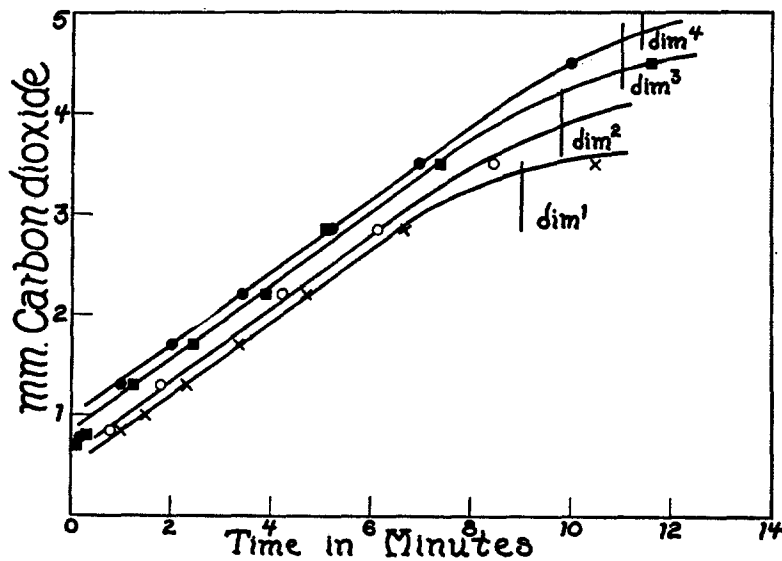


FIG. 2. Carbon dioxide production in a suspension of luminous bacteria. (Four determinations.)

to near the point of dimming, as one should expect if luminous bacteria consume oxygen independently of the concentration down to a low value of oxygen pressure. Before the point of dimming a slowing of the rate of carbon dioxide production occurs, comparable to the decrease of oxygen consumption following the lowering of oxygen to a point permitting incomplete activity of the respiratory mechanism of the cell.

In Fig. 2 a progressive increase in the time required for the dimming of a suspension of bacteria is indicated. Bacteria present in the



suspension gradually die and reduce the number of active cells. This will in turn decrease slightly the rate of oxygen consumption, allowing a longer time to be required for the reduction of the oxygen content to a point permitting dimming of the suspension because of lack of oxygen. An actual decay of the luminescence also occurs, even when the cells are adequately aerated. Hence, samples from the same suspension when repeatedly used for determinations will show a progressive

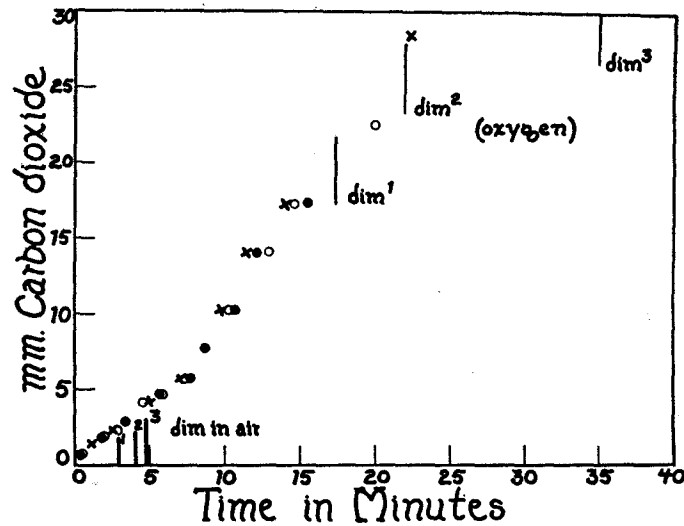


FIG. 3. Carbon dioxide production by luminous bacteria saturated with air and pure oxygen. (Three determinations.)

increase in the time for dimming. In very old suspensions a dim glow will be maintained for a remarkably long time.

Samples for carbon dioxide determinations were taken from a stock suspension that was at all times kept in equilibrium with the air and free of accumulating carbon dioxide by a stream of air continually passing through. If this stream of air is replaced by pure oxygen, it is possible to obtain a complete saturation of the suspension with pure oxygen instead of air. This was done in some cases, bringing the suspension into equilibrium with five times the amount of oxygen present in air. A five-fold increase in the time required for the dimming of the suspension would then be expected if the bacteria consume

oxygen at a constant rate independent of the pressure down to a low value of oxygen. Carbon dioxide should be produced in large quantities if all the oxygen in the suspension is consumed. This is approximately what does occur, as illustrated in Fig. 3.

Stephenson and Whetham<sup>29b</sup> have measured the carbon dioxide production of *Bacillus coli communis* when in equilibrium with air and oxygen and have obtained similar results.

*Relative Time for the Dimming of a Suspension of Luminous Bacteria Saturated with Air and Oxygen*

Determination	No. XII (air)	No. XII (saturated with oxygen)
1	3.10 minutes	17 minutes
2	4.05 "	22 "
3	5.00 "	35 "

It will be seen that in the last determination the time for dimming is *more* than five times that required for the dimming when in equilibrium with air. This is due to the reduced respiration of the bacteria when in contact with a very high concentration of oxygen, and to possible injury by high tensions of oxygen as indicated by Adams<sup>1</sup> and also by my own manometric experiments.

In every instance following carbon dioxide determinations in the above experiments, the suspension of luminous bacteria returned practically to its original pH on aeration.

*The Effect of Oxygen Tension upon Oxygen Consumption as Determined by Manometric Methods*

In the experiments of Callow<sup>6</sup> respiration of bacterial suspensions was successfully measured by manometric methods. In the present experiments the Thunberg-Winterstein microrespirometer was used in the form designed by Fenn,<sup>7a</sup> with additional modifications to permit the passage of a gas mixture into the respiratory chamber, directly above the suspension of bacteria.

By the use of a system of flow-meters, a mixture of pure nitrogen with air can be made in such a fashion that it is possible to obtain practically any desired oxygen concentration in the total mixture of gas. Pure nitrogen was obtained by passing the commercial gas over hot copper in an electric furnace. The gas was

conducted to the flow-meter through a tight system of glass and lead tubing, sealed at all joints with DeKhotinsky cement. Air was led from a second flow-meter and the gases mixed at a stop-cock connecting the two flow-meters and carried through a system of lead tubing to the water-bath containing the micro-respirometer. A complete diagram of the system is shown in Fig. 4. There was no leakage of gas or possible diffusion of oxygen into the system at any point. The only rubber connection was made very thick and extended only 2 mm. between the lead tubing and the glass tubing of the respirometer. The flow-meters were carefully calibrated for air and pure nitrogen, adjustments in pressures of the manometers giving very definite rates of flow through the capillaries of the flow-

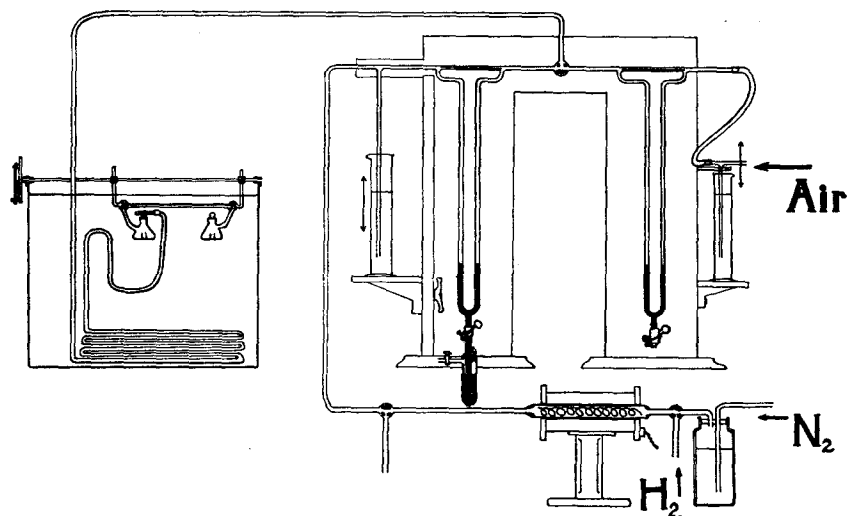


FIG. 4. Diagram of complete apparatus for determination of oxygen consumption with various gas mixtures containing partial pressures of oxygen, together with an arrangement for purification of gases.

meters. In the flow-meter conducting pure nitrogen, even the fluid of the manometer was thoroughly shaken with the pure gas before each experiment to drive off dissolved oxygen. At the microrespirometer the gas was brought into equilibrium with the bacterial suspension by a thorough shaking of the entire instrument with the mixture of gas passing through.

It was possible to make quick changes from one gas mixture to another and satisfactory determinations of oxygen consumption were easily made by this method. In each case a preliminary measurement of the rate of oxygen consumption of the particular suspension of bacteria was made in air before the suspension was subjected to a gas mixture containing only a low partial pressure of oxygen. After determinations of the rate of oxygen consumption in one or two gas

mixtures, the suspension was again brought into equilibrium with air and a check determination made to indicate if there had been a loss of respiratory activity due to decrease in the number of active cells during the course of the experiment. In each case a return to the original rate in air was made by the experimental suspension of the organisms. The suspensions were greatly diluted by phosphate buffer solution of pH 7.0, and brought into equilibrium with air by thorough shaking before a sample (1 cc.) was introduced into the respiratory chamber of the microspirometer. The whole apparatus was rocked mechanically in the water-bath, the temperature change varying no more than 0.2 of a degree during 8 hours. The speed of rocking made no difference in the rate of movement of the indicator

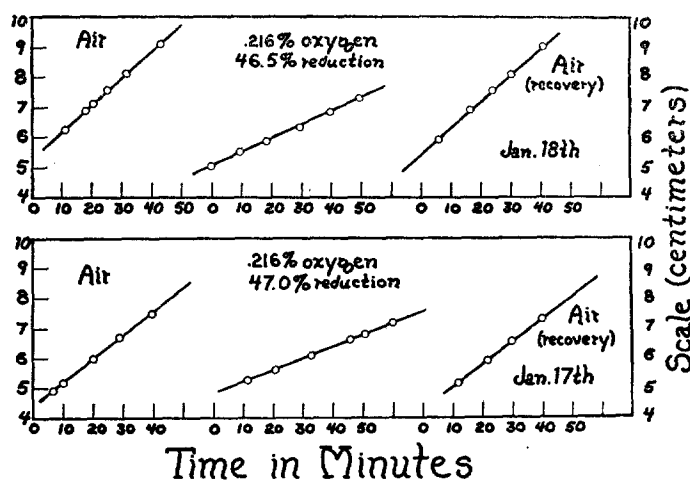


FIG. 5. Two microspirometer determinations of oxygen consumption with equal partial pressures of oxygen.

drop of kerosene in the capillary, so long as enough movement was maintained to keep the bacterial suspension in equilibrium with the gas mixture as determinations were being made.

When gas mixtures containing the same partial pressure of oxygen were brought into equilibrium with different suspensions of bacteria, respiring at different rates in the respirometer, the percentage reduction of the respiration was nearly identical for the same gas mixtures, although the total amount of oxygen consumed by the two suspensions differed widely. This occurred in every case. An example of two determinations with different suspensions of bacteria, but identical gas mixtures is given in Fig. 5. The partial pressure of oxygen in the

gas mixtures was 1.64 mm. Hg (0.216 per cent). The reduction of respiration in the two cases is in almost exact agreement. Equally close determinations with other suspensions and other gas mixtures were made, although the agreement was less marked when only very minute traces of oxygen were present.

The value of 0.26 per cent by volume or 1.97 mm. Hg partial pressure of oxygen has been given as the point at which luminescence of luminous bacteria just begins to dim. This is in agreement with observations made during respiration in the present experiments.

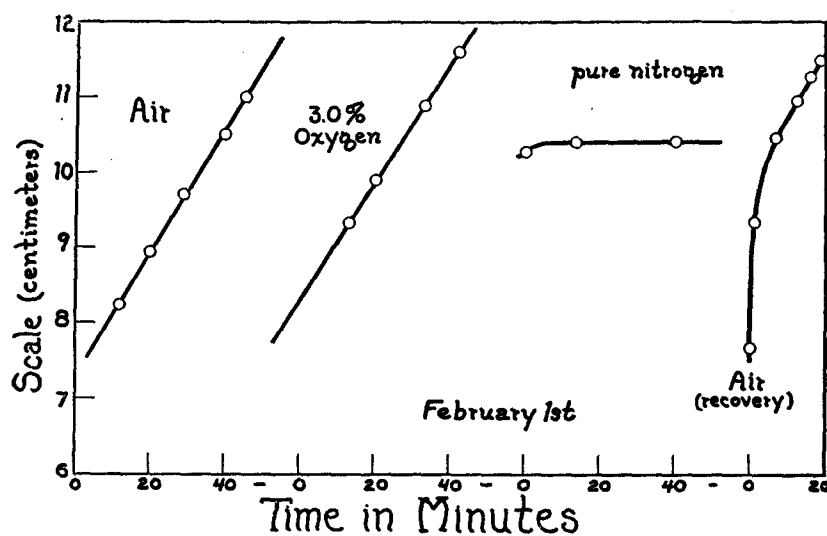


FIG. 6. Effect of pure nitrogen on oxygen consumption by luminous bacteria as measured in the microrespirometer.

When a gas mixture containing a partial pressure of oxygen equivalent to that in air (152 mm.) is brought into equilibrium with a bacterial suspension in the respirometer, there will be no increase in the rate of oxygen consumption over that at 22.80 mm. oxygen. If, however, the bacterial suspension is brought into equilibrium with pure oxygen, the rate of oxygen consumption will greatly diminish and no recovery to the original rate occurs when the suspension is again returned to air. The cells are irreparably injured by very high pressures of oxygen. This is in general agreement with the results

obtained in experiments on carbon dioxide production in equilibrium with pure oxygen.

When the bacterial suspension is brought into equilibrium with pure nitrogen, the respiratory activity quickly ceases, and no recovery occurs until the suspension is again brought into contact with air, whereupon an oxygen debt is indicated as the drop in the capillary of the instrument will move rapidly across the scale, finally falling in rate of movement to that previously obtained in air.

It has been indicated previously that after luminous bacteria have been in the absence of oxygen for some time, upon being readmitted to air they will luminesce with increased brightness for a very short period. This has occurred at the moment the bacterial suspension in the respirometer has been returned to air, and an increased movement of the drop indicates a portion of the greatly increased rate of oxidation following a period in the absence of oxygen.

From the equation for calculating adequate oxygen requirement for nerve given by Gerard<sup>8</sup> and Fenn,<sup>7a</sup> Harvey, in a recent paper<sup>11c</sup> has given the following relation for a bacterium  $1.1\mu$  in diameter, and  $2.2\mu$  in length:

$$C_o = \frac{Ar^2}{5D}$$

for the calculation of the oxygen pressure necessary to permit oxygen supply throughout a short cylinder such as a luminous bacterium, when  $A$  is oxygen consumption of the bacteria in cubic centimeters of oxygen per gram of bacteria per minute,  $r$  is the radius of the cylinder, and  $D$  is the diffusion coefficient for oxygen for the bacteria in cubic centimeters of oxygen diffusing per square centimeter with a pressure gradient of 1 atmosphere per centimeter, the assumption being made that oxygen consumption is independent of oxygen concentration at every partial pressure of oxygen. The calculated value for  $C_o$  obtained from Harvey's (1928) previous measurements of respiration comes out  $1.53 \times 10^{-5}$  atmosphere\* of oxygen at the surface of the bacterium to maintain adequate respiration. This theoretical value

\* Dr. Harvey informs me that this value,  $1.53 \times 10^{-5}$  atmosphere, should not have been corrected for solubility of oxygen in sea-water as was done in his paper.

is indeed far from the actual value, for as indicated in the curve for per cent of respiration in Fig. 7, the *observed* limiting value for adequate respiration is near 0.03 atmosphere, or 22.80 mm. Hg oxygen. All pressures above this value to equilibrium with air, permit a constant and maximum rate of oxygen consumption and at every partial pressure below this value there occurs a decrease in respiratory rate. Oxygen consumption is not independent of oxygen pressure over the

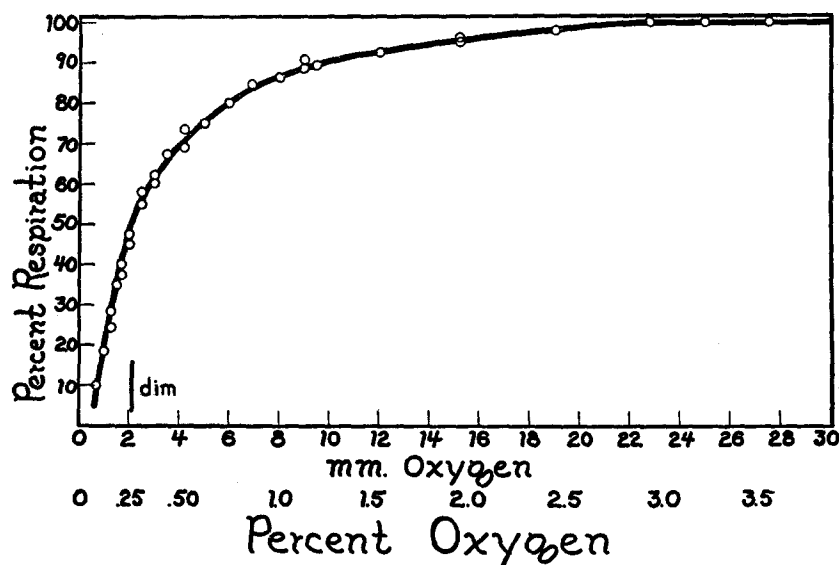


FIG. 7. The effect of oxygen tension upon oxygen consumption as measured by manometric methods.

whole range of respiratory activity, and the formula does not apply to luminous bacteria because of this assumption in the equation that oxygen consumption is always independent of oxygen pressure. The luminous bacteria are so small that the oxygen collecting at the catalytic surface of the oxidation mechanism of the cell becomes the limiting factor determining the rate of oxygen consumption rather than the oxygen diffusing into the cell.

When the partial pressure of oxygen in the gas mixtures in equilibrium with the suspension of bacteria are lowered from a value for adequate respiration, the decrease in respiratory activity does not

diminish in proportion to oxygen pressure over the whole range of oxygen concentrations. Oxygen consumption becomes about proportional to oxygen pressure only at low concentrations of oxygen following the dimming of a suspension of bacteria, when the respiration rate is reduced one-half. The curve for the reduction of respiration suggests adsorption of the gas in solution at a catalytic surface, reaching a saturation at approximately 22.80 mm. oxygen (0.03 atmosphere), at which an adequate and constant rate of respiratory activity is maintained. This is indeed similar to what has been observed in the case of inorganic catalysts, adsorbing a unimolecular layer of a gas, whose surfaces become unable to adsorb more molecules when once the surface capable of adsorption activity is covered. The per cent of maximum respiration of the bacteria will depend on the fraction of the oxidative catalyst of the cell covered by adsorbed molecules of oxygen, which in turn will depend on the rates at which oxygen is adsorbed and freed from the surfaces with the changing partial pressure of the oxygen in solution about the cells.

The curve for the reduction of respiration with decrease in oxygen pressure (Fig. 7) is exactly similar to well-known curves for the amount of gas adsorbed on catalytic surfaces which evaporate molecules with decrease in pressure.

Langmuir<sup>18a</sup> has discussed the relation of the extent of adsorption at various pressures of gas on a uniform surface capable of no further adsorption activity when completely covered by a unimolecular layer of adsorbed molecules, but which frees molecules of gas from the surface with decrease of pressure in agreement with an equilibrium:

$$k_1 p (1 - \theta) = k_2 \theta$$

when  $\theta$  is the fraction of adsorbing surface covered,  $(1 - \theta)$  the fraction of surface bare of adsorbed molecules,  $p$  the gas pressure, and  $k_1$  and  $k_2$  velocity constants characteristic of rates of condensation and evaporation from the surface respectively. If  $\theta$  is considered as per cent of respiration in the curve of Fig. 7, and  $(1 - \theta)$  as per cent reduction of respiration, the same relations hold, and  $\theta$  will be nearly proportional to the pressure only at low pressures of oxygen, and practically independent of the pressure near saturation of the oxidative catalyst of the cell, at which point the reaction becomes one of "zero-order,"



perfectly independent of increasing gas pressures. At this high pressure of oxygen near saturation,  $(1 - \theta)$  will be of small value, but will vary inversely as the pressure. The curve in Fig. 7 is a typical adsorption type, and is of the same form as curves from the data of Langmuir for adsorption of oxygen and nitrogen at mica and glass surfaces, and agrees with the form of those given by Pease<sup>25</sup> for adsorption of a number of gases at copper surfaces. I regard the data obtained for oxygen consumption at various pressures as supporting the view that the respiratory catalysts in the luminous bacteria are acting in a manner similar to those used in inorganic oxidations.

#### SUMMARY

1. The respiration of luminous bacteria has been studied by colorimetric and manometric methods.

2. *Limulus* oxyhaemocyanin has been used as a colorimetric indicator of oxygen consumption and indicator dyes were used for colorimetric determination of carbon dioxide production.

3. The Thunberg-Winterstein microrespirometer has been used for the measurement of the rate of oxygen consumption by luminous bacteria at different partial pressures of oxygen.

4. The effect of oxygen concentration upon oxygen consumption has been followed from equilibrium with air to low pressures of oxygen.

5. Luminous bacteria consume oxygen and produce carbon dioxide independent of oxygen pressures from equilibrium with air (152 mm.) to approximately 22.80 mm. oxygen or 0.03 atmosphere.

6. Dimming of a suspension of luminous bacteria occurs when oxygen tension is lowered to approximately 2 mm. Hg (0.0026 atmosphere) and when the rate of respiration becomes diminished one-half.

7. Pure nitrogen stops respiratory activity and pure oxygen irreversibly inhibits oxygen consumption.

8. The curve for rate of oxygen consumption with oxygen concentration is similar to curves for adsorption of gasses at catalytic surfaces, and agrees with the Langmuir equation for the expression of the amount of gas adsorbed in unimolecular layer at catalytic surfaces with gas pressure.

9. A constant and maximum rate of oxygen consumption occurs in

small cells when oxygen concentration becomes sufficient to entirely saturate the surface of the oxidative catalyst of the cell.

I wish to express my great debt to Prof. E. N. Harvey who first suggested this problem, for his advice, criticism, and encouragement, and also to Dr. R. N. Pease, of the Department of Chemistry, Princeton University, for some information regarding adsorption phenomena.

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