

TEMPERATURE CHARACTERISTICS FOR THE
METABOLISM OF CHLORELLA

II. THE RATE OF RESPIRATION OF CULTURES OF CHLORELLA
PYRENOIDOSA AS A FUNCTION OF TIME AND OF
TEMPERATURE

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(Accepted for publication, April 25, 1934)

I

Emerson (1926-27) has demonstrated the presence of two types of respiration in *Chlorella pyrenoidosa* and other green algae. The first is the normal respiration of the plant during the course of which a photosynthetic product is consumed, and is exhibited when a culture is suspended in Knop's solution. Iron-binding inhibitors such as cyanide slightly increase its rate. The second type of respiration is apparent on the addition of glucose to the suspending medium, when the respiration of the organism is doubled. The mechanism responsible for this increase differs from that concerned with the normal respiration, however, in that it may be inhibited by the action of cyanide.

In a previous paper (Crozier, Tang, and French, 1934-35) dealing with *Chlorella*, the temperature relations were determined for respiration in Knop's solution to which glucose had been added. We now consider the "normal" respiration of the organism, in order to determine the time course of the reaction at constant temperature, the respiratory quotient, and the temperature characteristic. We shall discuss these topics in the order mentioned. It is to be noticed that the conditions of these observations are such that constant rates of respiration are not to be expected. The cells have been actively and continuously engaged in photosynthesis, but with the beginning of the experiment they are transferred to darkness; metabolism then involves a declining store of photosynthetic reserves.

II

Two series of experiments were made without added glucose, one on June 1-3 (C.S.F.-P.S.T.), and one on Oct. 13-16 (C.S.F.-H.I.K.), 1933. The technique was essentially the same in both series, differences of detail being given in Table I.

The cells were from the same strain of *Chlorella pyrenoidosa* previously used, which were given us by Mr. William Arnold. They were grown in Knop's solution as previously described, 30 cm. from three

TABLE I
Data for Experiments of Series I and II on Respiration of Chlorella

| Series | I | II |
|--|---|--|
| Date of experiment | June 1 | Oct. 13 |
| Temperature of growth | 22°C. | 20°C. |
| Age of cells | 5-6 days | 10 days |
| Dry weight of cells per vessel | 19.5 mg. | 6.3 mg. |
| Number of flasks mixed | 6 | 4 |
| Approximate volume of cells per vessel | 121 mm. ³ | 48 mm. ³ |
| Temperatures | 0.9°, 5.9°, 10.9°, 15.9°, 21.0°, 28.0° | 0.6°, 3.5°, 8.5°, 13.4°, 18.5°, 23.4° |
| Duration of experiment | 47 hrs. | 60 hrs. |
| CO ₂ | Not measured | Measured |
| Rate with 1 per cent dextrose | Not measured | Measured at 3.5° and 18.5° |

50 watt Mazda bulbs; 5 per cent CO₂ in air was slowly bubbled through the flasks.

The measurements were carried out in conical Warburg vessels shaken at about 70 oscillations per minute, a 10 per cent solution of potassium hydroxide to absorb carbon dioxide partly filling the inset in those vessels by means of which we desired to study the oxygen consumption. Six tanks at different temperatures were operated simultaneously in dark rooms, there being two experimental vessels and one thermobarometer in each for the oxygen determinations. All the vessels within one series were filled from the same suspension to make the results directly comparable. At the higher temperatures fresh air was occasionally drawn through the vessels to avoid excessive reduction of the partial pressure of oxygen.

The carbon dioxide production was measured simultaneously, in Series II, by placing an extra vessel containing cell suspension but no hydroxide in each tank. Assuming the oxygen consumption within these extra vessels to be the same as in the vessels containing hydroxide, the carbon dioxide production may be determined by the formula,

$$(1) \quad x_{\text{CO}_2} = hK_{\text{CO}_2} - \frac{K_{\text{CO}_2}}{K_{\text{O}_2}} x_{\text{O}_2}$$

when

x_{O_2} = mm.³ O₂ per hour per 2 cc. of suspension measured by a vessel containing KOH,

x_{CO_2} = mm.³ CO₂ per hour per 2 cc. of suspension,

K_{O_2} = Vessel constant for O₂ for the vessel *without* KOH,

K_{CO_2} = Vessel constant for CO₂ for the vessel *without* KOH,

h = mm. increase in pressure per hour in vessel without KOH.

The simpler formula for the determination of oxygen consumption was given in the preceding paper.

III

The course of oxygen consumption as a function of time in several representative experiments at various temperatures is shown in the integral curves of Fig. 1, where for each vessel the total oxygen consumption in mm.³ per mg. dry weight is plotted against time. An inspection of the figure shows clearly that the time course of the reaction may be divided into two parts; a first portion, during which the rate is a function of time for about 25 hours, and a second, during which the rate is independent of time. Similar results are obtained for carbon dioxide production. That the change in rate during the initial phase is not due to centrifuging, or to the use of fresh Knop's solution, was proved by obtaining the same type of curve when cells in their growth medium were transferred directly to the respirometers.

A simple explanation for the decline in rate exhibited by the curves in Fig. 1 postulates that two substances, *A* and *B*, are oxidized during the initial phase, but only *B* during the final. This, of course, implies that the available supply of *A* is exhausted when the final rate is

exhibited. The behavior of the respiratory quotient, determined for various times at any given temperature, also suggests this, since it declines steadily from approximately 0.95 at the initial rate to the vicinity of 0.65 for the entire period of the final. Thus the ratio of

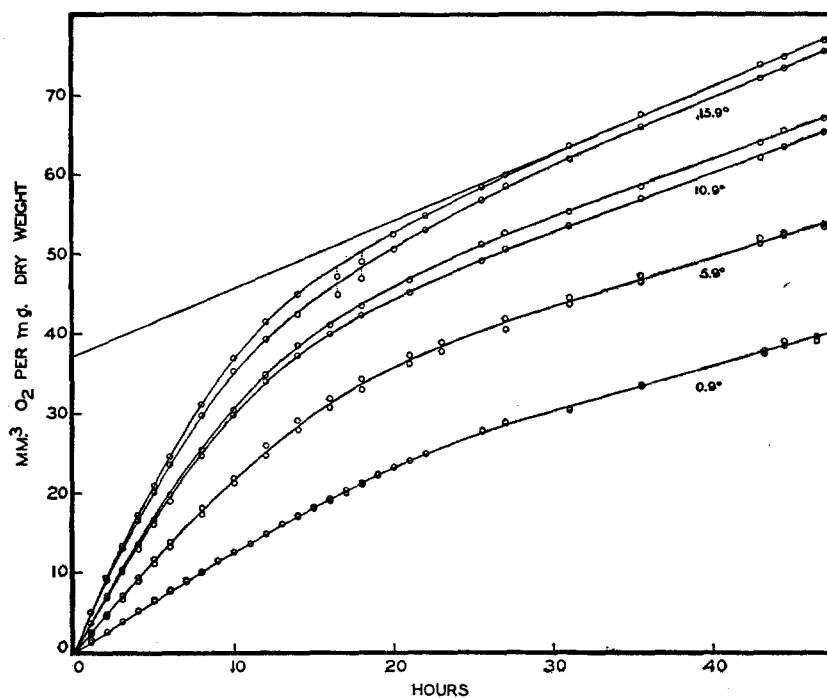


FIG. 1. Typical curves obtained when total oxygen consumed, in mm.^3 per mg. dry weight, is plotted against time. Duplicate determinations are graphed for each temperature, but only at higher temperatures are separate curves drawn for each. The amount of substance A , in mm.^3 of oxygen used by it, is given by the value of the intercept obtained when the final constant rate is projected to the y -axis at $t = 0$.

respired substrates, A/B , decreases in value during the entire initial phase, when we assume A to have a respiratory quotient of 1 and B of 0.65. This assumption will be justified later. Table II gives the initial and final values of the respiratory quotient for those temperatures at which we determined the carbon dioxide production. It

appears that the initial and final values of the quotient are independent of temperature.

In addition to the hypothesis that two substances are oxidized during the normal respiration of *Chlorella*, the curves of Fig. 1 and the behavior of the respiratory quotient suggest that in the case of substance *A* its own concentration is the factor limiting the rate of oxygen consumption, and we may be able to describe the rate of its oxidation by some simple differential equation. If this equation be first order, the rate of oxygen consumption should be a linear function of the amount of oxygen consumed until the value of the respiratory quotient becomes constant (*i.e.*, until the final rate is established). In this we

TABLE II
Initial and Final Values of the Respiratory Quotient for Chlorella pyrenoidosa at Various Temperatures

| Temperature | Initial r. q. | Final r. q. |
|-------------|---------------|-------------|
| °C. | | |
| 0.6 | 0.87 | 0.65 ± 0.05 |
| 3.5 | 0.98 | 0.60 ± 0.04 |
| 13.4 | 1.00 | 0.70 |
| 18.5 | 0.94 | 0.65 ± 0.05 |
| 23.4* | 0.93 | 0.70 |

* Additional experiment made to determine this quotient.

assume that the concentration of *A* is inversely proportional to the amount of oxygen utilized.

In Figs. 2 and 3 the first order equation has been tested graphically by plotting the rate of oxygen consumption in mm.³ per vessel per hour against mm.³ of oxygen consumed. It will be observed that the curves at higher temperatures approximate a linear function during the initial phase, but as the temperature decreases a pronounced deviation becomes apparent. Presumably, some reaction not involving oxygen modifies the amount of *A* available for respiration, and since its effect is greater at lower temperatures, the value of the temperature coefficient for the modifying reaction must be less than that for the oxidation of *A*.

Another chain of reasoning strongly suggests the presence of this

additional reaction. The apparent initial concentration of A may be measured in terms of the total amount of oxygen consumed by its oxidation. This quantity may be determined from the integral

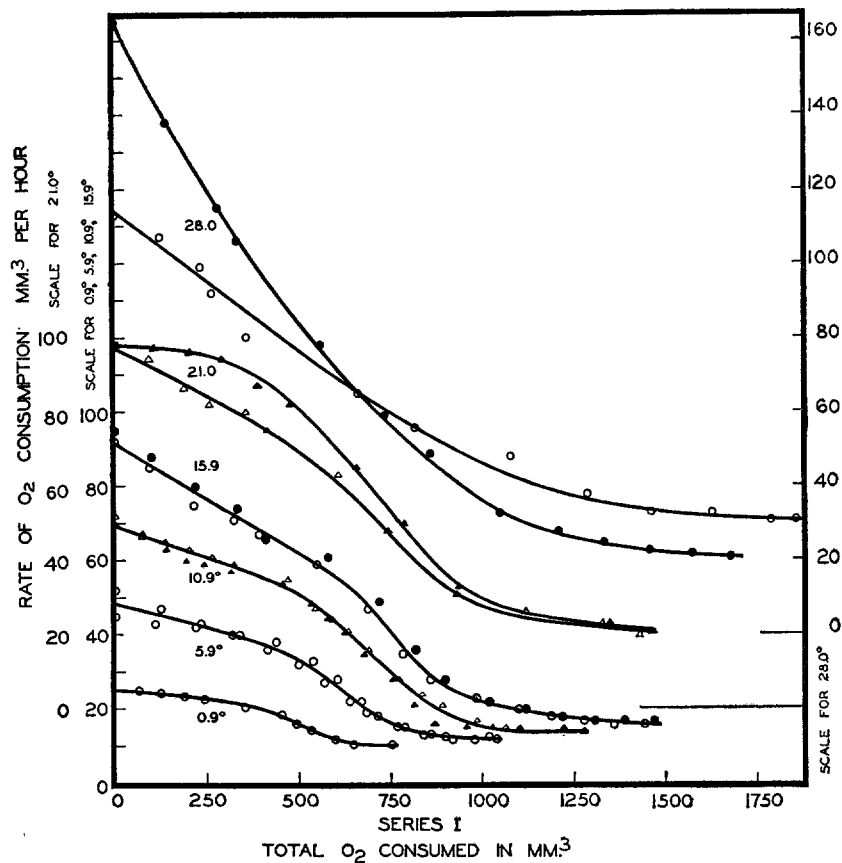


FIG. 2. Rate of oxygen consumption, in mm.^3 per vessel per hour, plotted against total mm.^3 of oxygen consumed, for Series I. Zero on the y -axis has been raised for two of the curves; that for 21.0°C . is located at 20 mm.^3 , for 28.0°C . at 40 mm.^3 on the scale of the lower temperature curves. The base lines of these two are indicated by horizontal lines at the right.

curves, some of which are shown in Fig. 1, which represent the amount of oxygen consumed as a function of time. The straight line portions of the curves, indicating the constant rate during the final period

due to the oxidation of substance *B*, are continued so as to intercept the *y*-axis at zero time. The value of this intercept is the amount of oxygen consumed by *A* during the initial phase, since the extrapolation automatically subtracts the amount of oxygen consumed by the oxidation of *B* during this period. The results are shown in Fig. 4, where

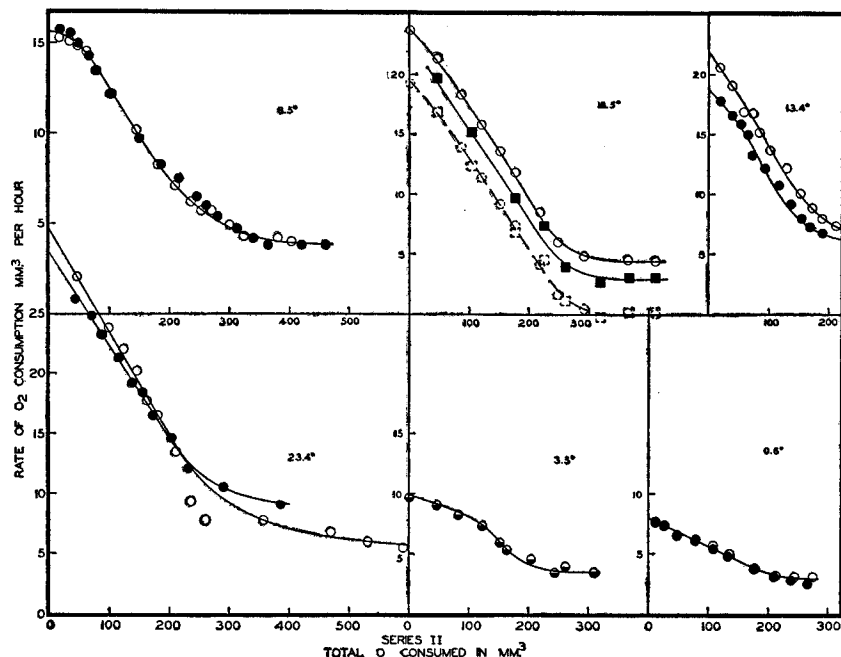


FIG. 3. Rate of oxygen consumption, in mm.³ per vessel per hour, plotted against total mm.³ of oxygen consumed, for Series II. Duplicate determinations are shown for all experimental temperatures except 18.5°C. Instead, at the latter, the following are plotted against total oxygen consumed; carbon dioxide production (solid squares), oxygen consumption and carbon dioxide production due to the oxidation of substance *A* (dotted points).

the amount of *A*, in terms of mm.³ of oxygen used by it per mg. dry weight, is plotted as a function of the Centigrade temperature. The solid circles are for Series I, the open circles for Series II. The figure states that the total amount of *A* is a function of temperature, which certainly cannot be so. Again, therefore, we are led to infer the existence of a reaction depleting the amount of *A* available for respira-

tion, and since the process must have a low temperature coefficient, we suggest that it is of a simple, physical nature, such as diffusion.

Previously, we have stated that the two substances, *A* and *B*, apparently involved in the normal respiration of *Chlorella* have respiratory quotients of 1.00 and 0.65 respectively. The evidence upon which this conclusion is based is derived from an analysis of the curves

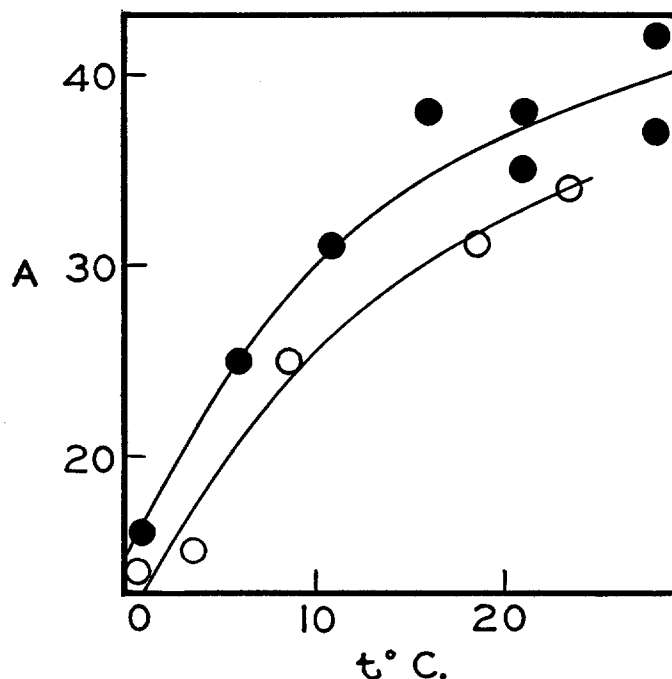


FIG. 4. Apparent initial amount of substance *A*, expressed in terms of total volume of oxygen consumed by its complete oxidation, plotted against the Centigrade temperature. Solid circles indicate Series I, open ones Series II.

in Figs. 2 and 3, where the rate of oxygen consumption is plotted against total oxygen consumed. When the carbon dioxide data are treated likewise, curves paralleling those for oxygen are obtained; this is illustrated by the curves for 18.5°C. of Series II in Fig. 3, where the circles indicate oxygen and the squares carbon dioxide. In either case, if we subtract the final rate from all rates which precede it, we obtain a curve dealing only with the oxidation of *A*. This has been

done in the case of the data for 18.5°C., the resulting curve being indicated by a broken line; it will be observed that the same curve fits the corrected points for both oxygen and carbon dioxide—in other words, that the respiratory quotient is 1.00. The nearly linear relationship obtained in this case is due to the relatively high temperature, but the respiratory quotient is independent of temperature (see Table II). Since the respiratory quotient of *B* (0.65), determined from the final rates, likewise is independent of temperature, we conclude that two substances are used in respiration. Though we cannot specifically identify them, the quotients imply that *A* must have the general formula, $C_xH_{2x}O_x$, and *B* must be some relatively oxygen-poor substrate such as an alcohol or fat.

IV

The fact that two substances may be involved in the normal, cyanide-stabile respiration of *Chlorella pyrenoidosa* raises two questions: first, are one or more enzyme systems involved, and secondly, can their identity be distinguished from that of the glucose-respiration complex by evidence other than that from experiments involving cyanide poisoning? To answer these we offer the results of our temperature data.

Experimentally it has been found that the velocity constants of chemical reactions in general vary with temperature in an orderly manner. Equation 2 states this relationship:

$$(2) \quad \text{Log } K_1/K_2 = \mu/R \cdot (1/T_2 - 1/T_1)$$

when

K_1 = velocity constant at absolute temperature, T_1 ,

K_2 = velocity constant at absolute temperature, T_2 ,

R = gas constant (1.98),

μ = constant having the dimensions of calories per degree, and which has been designated variously as the energy of activation of the substrate (Arrhenius), and energy of activation of the catalyst (Rice).

Both of the above interpretations of μ agree in that each implies that the value of μ is specific for any reaction complex and relatively independent of temperature.

In particular, Crozier (1924–25) and Crozier and Stier (1924–25;

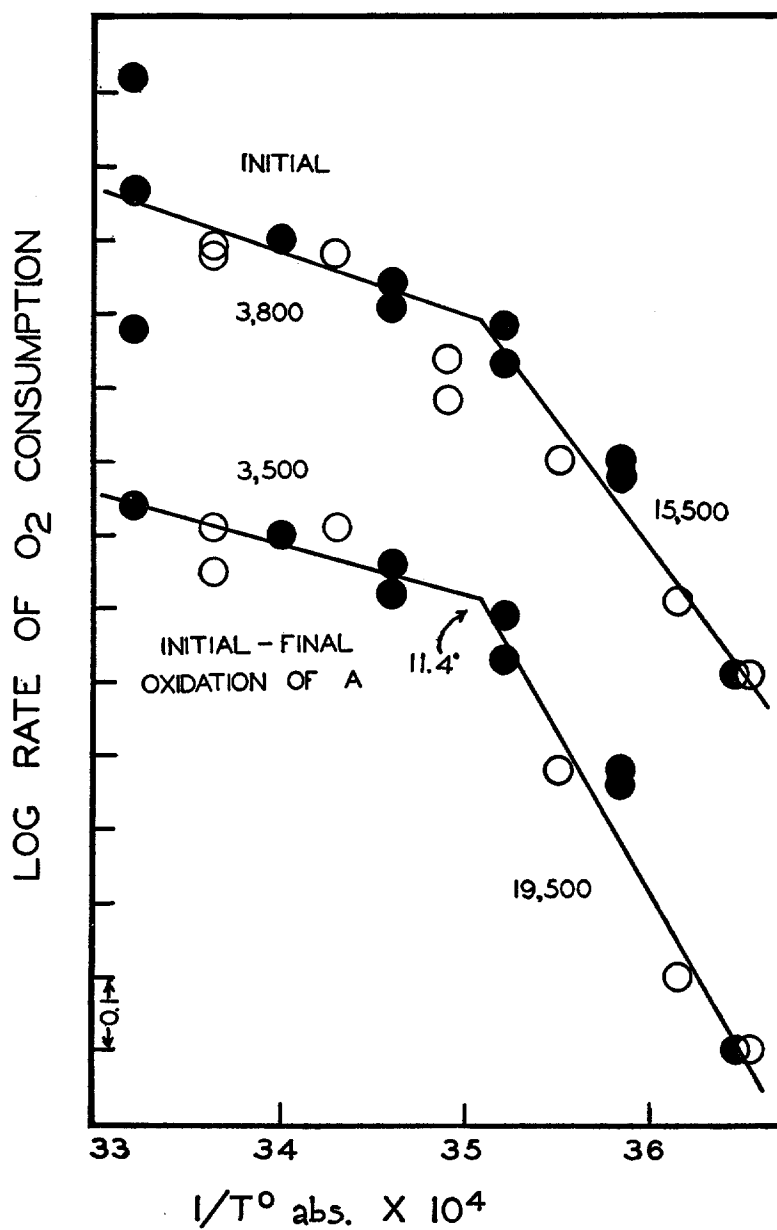


FIG. 5. Logarithm of initial rate of oxygen consumption due to oxidation of substance *A* plotted against reciprocal of absolute temperature (lower curve). For this process μ has a value of 3,500 calories above and 19,500 calories below a critical temperature near 11.5°C. For comparison, the plot for the initial rate (due to oxidation of *A* plus *B*) is shown (upper curve). Solid circles represent Series I, open circles Series II.

1926-27) have applied this equation to a considerable array of biological data in order to determine whether μ is a constant such as may be used to characterize a given physiological process. To determine μ , the natural logarithm of the rate is plotted against the reciprocal of the absolute temperature, and if a straight line may be drawn through the resulting points, the value of its slope is $-\mu/R$. On occasion

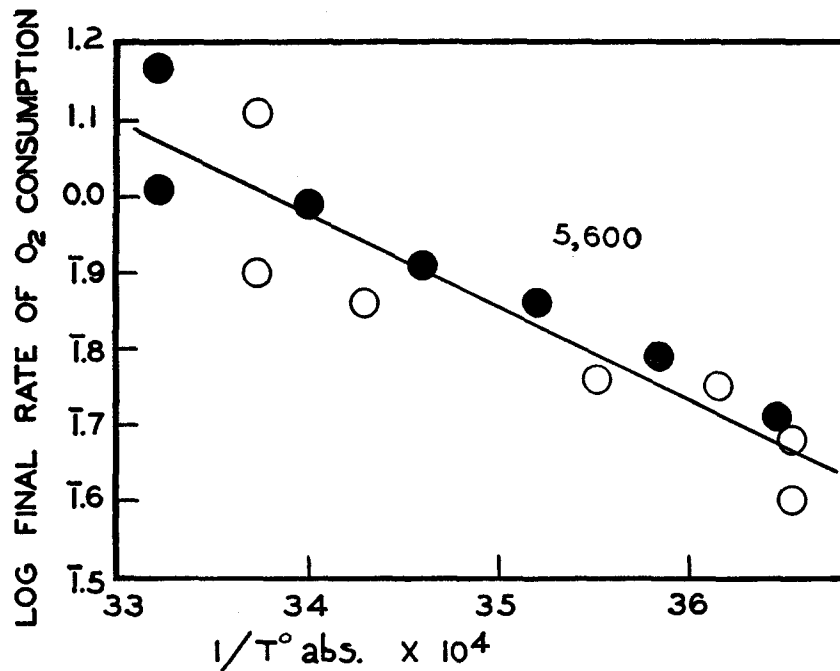


FIG. 6. Logarithm of final rate of oxygen consumption plotted against reciprocal of absolute temperature. The value of μ is 5,600 calories and is related to the oxidation of substance *B*. Solid circles for Series I, open circles for Series II.

it is necessary to draw two straight lines of different slopes for two temperature ranges through the experimental points. It is of interest to determine the values of μ for the oxidation of *A* and *B*, and to compare these with the values obtained for the glucose respiration and for the respiration of other organisms.

In Fig. 5 there are plotted the natural logarithms of the initial rate (rate of *A* plus *B*) of oxygen consumption and the initial rate for *A*

against the reciprocal of the absolute temperature. The former has been plotted merely for purposes of comparison, since its value of μ , depending upon the sum of two rates, is without theoretical significance. Unless we wish to fit the data with a curved line, the initial rate has two values of μ , 3,800 above and about 15,500 below a critical temperature in the neighborhood of 11.5°C. The initial rate of oxygen consumption by *A* gives μ values of 3,500 above and 19,500 below the critical temperature. Since the respiratory quotient does not vary with temperature, the value of μ for carbon dioxide production will be identical. The observations of Yabusoe (1924-25) when so plotted gives μ the value of 9,500 from 10 to 30°C. This is on the basis of determinations at but three different temperatures, however, and was made on *Chlorella vulgaris* instead of *pyrenoidosa*.

TABLE III
Rate of Oxygen Consumption by *Chlorella* with and without Glucose

| Temperature °C. | Glucose | Normal | Normal Glucose |
|--------------------|---------|--------|-------------------|
| 3.5° | 1.95 | 1.5 | 1.3 |
| 18.5° | 8.9 | 4.3 | 2.0 |

The μ plots for the final rates of oxygen consumption, which represent the rate of oxidation of *B*, are given in Fig. 6, where the solid circles represent Series I and the open circles Series II. The value of μ , 5,600, is constant over the entire temperature range, and applies to carbon dioxide production as well, since the final respiratory quotient is also independent of temperature.

As a check on previous experiments in which the glucose respiration of *Chlorella* was investigated, the rate of oxygen consumption in Knop's solution containing 1 per cent glucose was determined. The results at two temperatures, 3.5° and 18.5°C., are given in Table III. On the basis of these two points the value of μ is 19,000 calories, which agrees with that published previously.

In Fig. 7 the observed initial and final rates of oxygen utilization are plotted directly against the temperature. It is of interest that, in

data with this degree of scatter, the rates appear to be linearly related to the temperature.

v

Table IV summarizes values of μ obtained for several metabolic processes in *Chlorella pyrenoidosa*, and indicates that different processes

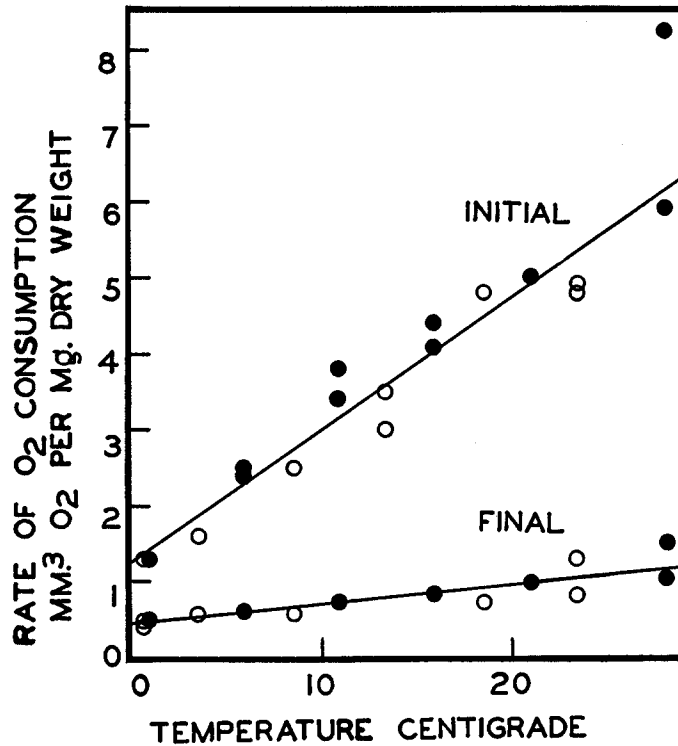


FIG. 7. The initial and final rates of O_2 consumption when plotted against temperature Centigrade give a straight line. Compare with Figs. 5 and 6, and see text.

within the same organism may be characterized by different values of this constant. The values of μ for the different types of respiration not only differ among themselves, but from that for photosynthesis as well. Since the values for the respiration of *A* and *B* differ, we have further evidence favoring the idea that the normal respiration of

Chlorella involves at least two different systems. For the determination of values of μ it is not wise to assume that the quantity measured is dependent upon a single reaction complex, unless an attempt has been made to check this assumption experimentally. In the case of our experiments, this would have meant the acceptance of the value of μ determined on the basis of the initial rate (*A* plus *B*), a value dependent upon the rates of two processes.

The value of μ obtained for the respiration of *A* below the critical temperature agrees with that for the glucose respiration (19,000),

TABLE IV
Values of μ Determined for Metabolic Processes in Chlorella pyrenoidosa

| Process | μ | | Critical temperature °C. |
|--|----------------------------|----------------------------|-----------------------------|
| | Above critical temperature | Below critical temperature | |
| Oxidation of <i>A</i> | 3,500 | 19,500 | 11.5 |
| Oxidation of <i>B</i> | 5,600 | | |
| Oxidation of <i>A</i> plus <i>B</i> (initial rate)..... | 3,800 | 15,500 | 11.5 |
| Photosynthesis | 13,000 ± 1,000† | | |
| Glucose oxidation..... | (12,000) | 19,000 | (15) |
| H ₂ O ₂ * decomposition by intact cells..... | 10,500 | | |

* French (1934-35).

† Unpublished data of W. A. Arnold and H. I. Kohn.

whereas above the critical temperature, it does not (3,500). The 19,000 value has been obtained for yeast by Stier (1932-33) between 3-15°C., and by Lineweaver, Burk, and Horner (1931-32) for *Azotobacter*.

The values of μ obtained for the oxidation of *A* above the critical temperature (3,500) and for the oxidation of *B* throughout the experimental temperature range (5,600) are the lowest yet recorded for a respiratory process. In part, this may be due to the fact that in temperature studies of respiration this process has not been sufficiently separated out from other concomitant processes.

SUMMARY

The respiration of the green alga *Chlorella pyrenoidosa*, suspended in Knop's solution, has been studied in the dark as a function of time and of temperature. The rates of oxygen consumption and of carbon dioxide production (at constant temperature) decline for about 25 hours to a low, constant level. From an analysis of the curves it is suggested that two substances, *A* and *B*, are utilized, whose respiratory quotients are 1 and 0.65 respectively. The values of the temperature characteristics were found to be: for oxidation of *A*, 19,500 (0.6 to 11.5°C.) and 3,500 (11.5 to 28°C.); for oxidation of *B*, 5,600 (23.4 to 0.6°C.).

It is a pleasure to thank Mr. William Arnold and Mr. C. P. Winsor for their advice and help.

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