TEMPERATURE CHARACTERISTICS FOR METABOLISM OF CHLORELLA

I. The Rate of O_2 Utilization of Chlorella pyrenoidosa with Added Dextrose

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I

The unicellular alga *Chlorella pyrenoidosa* has been extensively studied with respect to its photosynthetic activities (*cf.* Warburg, 1928; Emerson, 1928–29; Emerson and Arnold, 1931–32; Arnold, 1933–34). It exhibits a measurable rate of respiration in Knop solution without glucose; this respiration seems to differ from the more active respiration apparent when glucose is added to the medium (Emerson, 1926–27), as the two behave differently toward inhibitors (HCN, H_2S, CO). With this organism, then, it should be possible to obtain temperature characteristics (Crozier, 1924–25) for the velocities of the photosynthetic reactions, for several aspects of respiration, and for growth (multiplication). For these processes, also, the nature of the temperature relations may be investigated through the influence of alterations of the medium upon them. In this way desirable information may be secured as to the meaning of the temperature characteristic, μ, of the equation

$$\text{velocity} = ke^{-\frac{\mu}{RT}}$$

if the relationship is found to provide a satisfactory description of the measurements (*cf.* Crozier, 1924–25, *et seq.;* Stier, 1932–33). Data suitably obtained should then give some information as to the probability that the reproducible constant μ may have reference to a specific property of a relatively simple chemical system controlling the

rate of a measured process (*cf.* Crozier, 1924–25; Crozier, Stier, and Pincus, 1929; Pincus, 1930–31). We know that exposure to ultraviolet radiation may be used to induce decay of photosynthetic activity in *Chlorella* without simultaneous interference with respiration (Arnold, 1933–34). It may be found that critical temperatures and other features of the curves relating speeds of diverse respiratory activities of *Chlorella* to temperature are notably different. Such findings would be effective in support of the general conception that the kinetics of relatively uncomplicated controlling catalyzed processes in living cells may be adequately characterized in specific respects. We shall deal first with respiration (O_2 utilization) by *Chlorella* in Knop solution containing 1.0 per cent glucose.

II

A pure culture of *Chlorella pyrenoidosa* from the same stock as that used for studies on photosynthesis by Emerson and Arnold (1931–32) was kindly supplied to us. It was grown in Knop solution. The flasks (Warburg and Negelein, 1922) were about 300 ml. in capacity, glass-sealed except for the openings of the inlet and outlet tubes provided for the passage of a gas mixture. The latter was 5 per cent CO_2 in air, stored in a commercial gas cylinder and allowed to bubble very gently through the cultures. The flasks were placed in a large crystallizing dish 30 cm. above three 65 watt frosted lamps, with a stream of water passing constantly through the dish to keep the temperature surrounding the cultures at about $20° \pm 2°$.

The culture solution is the modified Knop solution as used by Emerson (1926–27). Stock solutions were made up of:

(*A*) $MgSO_4 \cdot 7H_2O$, 50 gm. per liter.
(*B*) KNO_3, 25 gm. per liter.
(*C*) KH_2PO_4, 25 gm. per liter.
(*D*) $Ca(NO_3)_2$, 11·3 gm. per liter.
(*E*) $FeSO_4 \cdot 7H_2O$, 0.336 gm. per liter.

Solution (*D*) was used here to make up for the amount of Ca present in the tap water used by Warburg and others. 50 ml. each of (*A*), (*B*), (*C*), (*D*), and 0.5 ml. of the $FeSO_4$ solution were mixed and diluted with distilled water to 1 liter.

Under the stated conditions of culturing, luxuriant growths of cells may be had in from 7 to 10 days, and cultures of that age were used throughout the experiments. Usually the contents of one flask, about 200 ml. of cell suspension, were used in a single series of experiments. The cells were centrifuged and washed twice with a freshly prepared Knop solution containing 1 per cent glucose (1 gm. in 100 ml.). The conditions of the centrifuging were: 2000 R.P.M. with a relative

gravitational force of 800 in a 100 ml. flask for 5 minutes at a time. Under such conditions, a culture 7 to 10 days old gives about 500 c.mm. of cells.[1]

A 2 ml. portion of the suspension containing about 20 mm.[3] of cells was pipetted into a conical vessel of the Warburg respirometer. The vessel used was about 15 ml. in capacity with a side arm and with an inset for alkali. In our experiments the inset contained 0.2 ml. of a 10 per cent NaOH. The vessels had been soaked in chromate-sulfuric acid mixture overnight, and were repeatedly rinsed with tap water, and then with distilled water; finally they were filled with distilled water and allowed to stand for at least 10 minutes. They were dried in an oven maintained at 105°. A ring of paraffin was smeared around the inner edge of the inset to prevent the creeping of the alkali into the main portion of the vessel. The vessels, after being connected to the manometers, were placed in thermostats the temperature of which may be maintained to within ±0.01° (described by Stier and Crozier, 1932–33). The respirometers were shaken at a speed of 70 complete oscillations per minute with a throw of 8 cm. Preliminary experiments showed that the rate of O_2 consumption was independent of shaking speed between 40 and 100 oscillations per minute. The experiments were performed in darkness and the readings were made with a weak neon lamp so arranged that the light fell on a spot on the manometers. At least 30 minutes were allowed for thermal adaptation at each temperature before the stop-cocks of the manometers were closed and the first readings taken. Readings were taken thereafter at about ½ hour intervals for at least 2 hours, and sometimes for 10 hours at a given temperature.

The temperature was varied in two ways. In certain series of experiments, two to four thermostats were operated at once, each at a different temperature, and the rates of O_2 consumption by equal quantities of cells from the same suspension were compared. In other series of experiments only one thermostat was used and the cells were subjected to successive changes of temperatures throughout the range used. For instance, in one series, the rates of O_2 consumption were observed for a certain length of time at 15°. Without disturbing the experimental set-up, the temperature of the tank was lowered to 10°. After a period of 30 minutes for thermal adaptation, during which the stop-cock of the respirometer was turned so that the system was open to the air, the cock was closed again and readings were resumed for another period. At the end of this period the temperature was lowered to 5.0° and the process was repeated. After a period of 5°, the temperature was raised to 7.5°, to 12.5°, then to 17.5°, 22.5°, and finally to 25°, and readings were made at these temperatures after sufficient time had been allowed for thermal adaptation.

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[1] The apparent volume of cells in a given suspension changes with conditions of centrifuging. The volumes obtained are given merely for rough comparisons, and should not be taken for computation of absolute rates.

III

It is necessary to show that at constant temperature the rate of respiration is sufficiently constant throughout the period of observa-

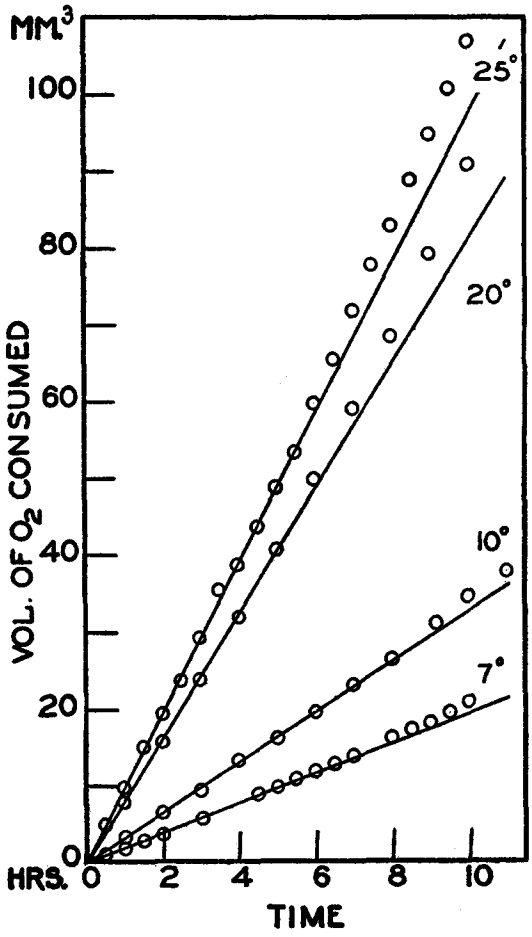

FIG. 1. Amounts of O_2 consumed by *Chlorella* cells are plotted against time; four typical experiments. The rate of utilization of O_2 is constant for about 5 hours.

tion (Crozier and Navez, 1930–31; Tang, 1930–31; Stier, 1932–33) and that it is not appreciably influenced by previous sudden changes in temperature.

A number of experiments were performed at various constant temperatures for about 10 hours to establish the constancy of rate for the duration of our experiments. Fig. 1 gives the results of four representative experiments, at 7°, 10°, 20°, and 25° respectively. The rates are constant for about 5 hours, after which they rise, due probably to increase in the number of *Chlorella* cells (or to growth of bacteria). Measurements made for shorter periods than 5 hours are then reliable within the errors of the method.

The controversial "stimulating" effect of alternating temperature on life processes has often been reported and discussed; Kostychev (1927) gives a review of this matter as it concerns respiration (*cf.* Crozier and Navez, 1930–31; Tang, 1930–31). To see whether sudden changes in temperature had any special effect upon O_2 consumption by *Chlorella*, a series of tests was made. Three thermostats

TABLE I

Effect of Sudden Changes in Temperature upon Rate of O_2 Consumption by Chlorella pyrenoidosa

Temperature change	n'	n	n''
18–13°	1.0	1.1	1.0
13– 8°	1.0	1.2	1.0
13–18°	1.0	0.9	1.0
8–13°	0.9	0.95	1.0

n' is the ratio of the rates of the cultures subjected to changing temperatures (hereafter called *E. C.*) to those of the control cultures (*C. C.*) in the first period, when they were at the same temperature; n is the ratio of the rates of *E. C.* to *C. C.* when the former were changed to a lower or higher temperature; n'' is the ratio of *E. C.* to *C. C.* when the former were returned to the original temperature in the third period.

were operated at 8°, 13°, and 18° respectively. Fourteen samples of *Chlorella* cells, all from the same suspension, were distributed as follows: four each at 8° and 18°, and 6 at 13°. After a period of respiration of about 2.5 hours, two cultures from the 13° group were transferred to 8° and two to 18°. In return, two cultures each from 8° and 18° were placed at 13°. After a second period of respiration, sufficient time being allowed for thermal adaptation in all cases, the cultures were again interchanged so that they were at the same temperatures as they had been at the beginning of the experiment. Thus we have a set of two control experiments at each temperature which respired at constant temperature for a long time, and two cultures which were first subjected to a given temperature, then to a lower or higher temperature, and were finally returned to the original temperature. In all cases, the rates for the cultures which were returned from a sojourn at a higher or lower temperature for a period of about 2.5 hours were very nearly the same as

those of the controls which remained undisturbed at the initial temperatures. The rate of O_2 consumption of a culture transferred from a higher temperature to

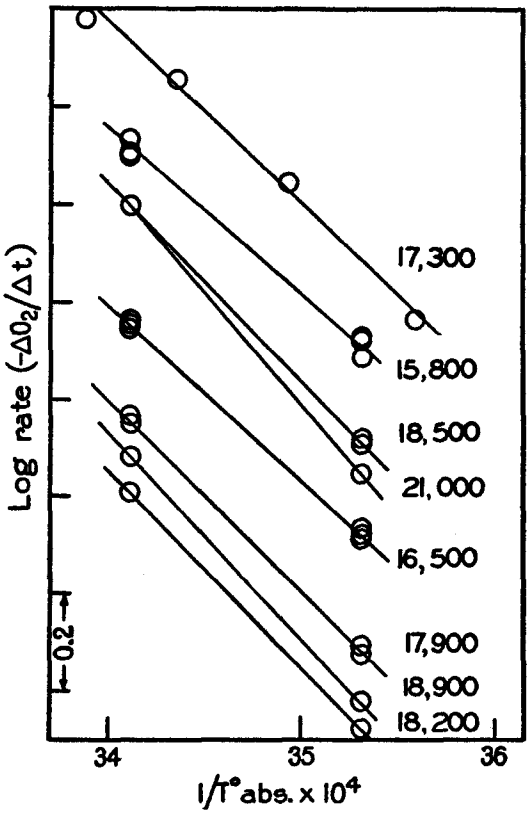

FIG. 2

FIGS. 2 and 3. Log relative rates of O_2 consumption by *Chlorella pyrenoidosa* is plotted against $1/T$ abs. $\times 10^4$. Each line represents a series of experiments. The order in which the points were taken is either marked by numbers attached to the groups of circles or by different symbols. In the latter case the solid circles represent the experiments made during the first period and the open ones are those made in the second period (see text). The values of μ are given along the individual lines; see text. Fig. 2 represents points taken from the data of Tang and French (1933–34) obtained in connection with measurements made at various O_2 tensions.

a lower is slightly higher than that of the control experiment maintained constant at the lower temperature. Conversely, when a culture is transferred from a lower

to a higher temperature, the rate is slightly lower than that for a culture at the higher temperature from the beginning. The differences are small, and but for their consistency appear to be within the limit of error. The results are summarized in Table I.

When proper precautions are taken, that is, when sufficient time has been allowed for thermal adaptation, and when the experiments are not performed over too long a period at a given temperature, the slight variation of the rates with time and with sudden changes in temperature may be considered negligible.

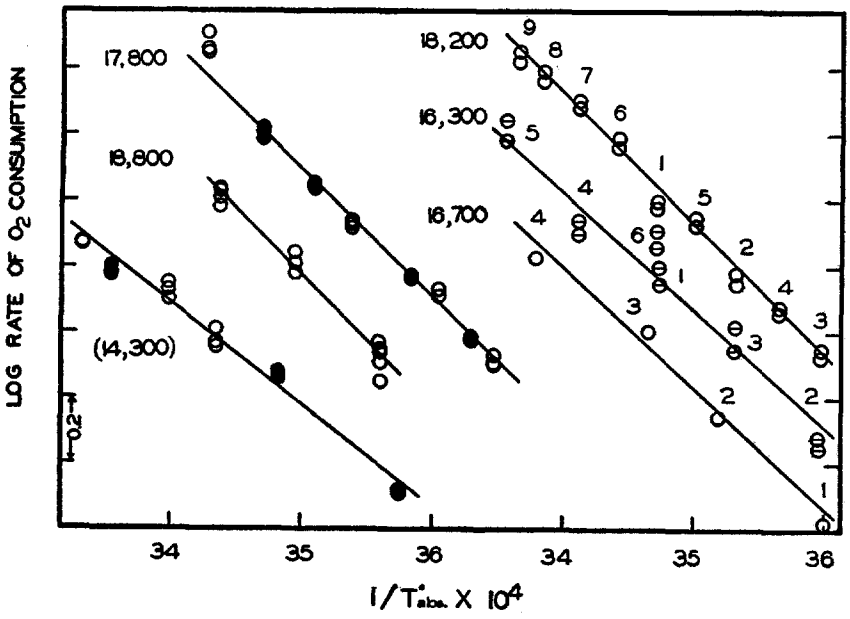

FIG. 3

IV

We may now consider the results obtained when *Chlorella* cells respire at temperatures ranging from 1° to 27°. Fourteen series of experiments were performed altogether, with the two methods already described. All the data are plotted individually in Figs. 2 to 4; the ordinates give the logarithms of the relative rates of O_2 consumption, the abscissae give $1/T$ abs. $\times 10^4$. Each series consists of 1, 2, or 3 experiments; all the points are plotted in the figures. Whenever neces-

sary the order in which the points were obtained is given by the numbers attached to the groups of points, or by the way in which the points are represented. In any given series, the solid circles are points obtained in the first period and the open circles represent points which were obtained at the second period. The values of μ, the temperature characteristic (Crozier, 1924–25), are given along the individual lines. It is seen that the values vary from series to series falling between 14,300 and 21,200.

Observations with *Chlorella* cultures, under the conditions we have described, are open to a number of sources of confusion. From much earlier work we know that determinations of μ from readings at two or three temperatures are subject to the possibility of a considerable uncertainty arising from the natural latitude of variation found in successive or repetitive determinations at the same temperature (*cf.* Crozier and Stier, 1924–25; Stier, 1932–33); this variation may be of different relative magnitudes on either side of a critical temperature (*cf.* Crozier and Stier, 1926–27; Stier, 1932–33). Even were the sometimes disturbing effects of the sensitivity (as contrasted with the accuracy) of the manometric method completely avoided, we cannot be certain that all the cells in a given culture will respond in the same manner to transitions from one temperature to another. For such reasons relatively considerable variation must be expected in the apparent value of μ computed from determinations at only two temperatures (*cf.* also Lineweaver, Burk, and Horner, 1931–32). It is therefore necessary to combine the several series of determinations, to obtain the most probable value of the temperature characteristic.

All the data for the fourteen series of experiments were brought together by shifting the lines in Figs. 2 and 3 to a common level at 15°C. by proper factors, giving the mass plot of Fig. 4. The points show a considerable degree of scatter, greater at higher temperatures. A line is drawn through the points indicating the general trend of the change of the rates with temperature. The slope of that line gives the value $\mu = 19{,}000$ cal. The points are relatively quite scattered, more so at the higher temperatures; this may be due, in part, to special effects at the higher temperatures, or to a real difference in the latitude of variation above a critical temperature at 20°±. The points do not fall within parallel lines, as they do in many other instances (*cf.*

Crozier *et al.*[2]); this indicates that technical errors enter in this case to a relatively large extent.

The highest individual value of μ is roughly 21,000, based, however, on two points only (Fig. 2); the lowest, 14,300, shown in Fig. 3, can-

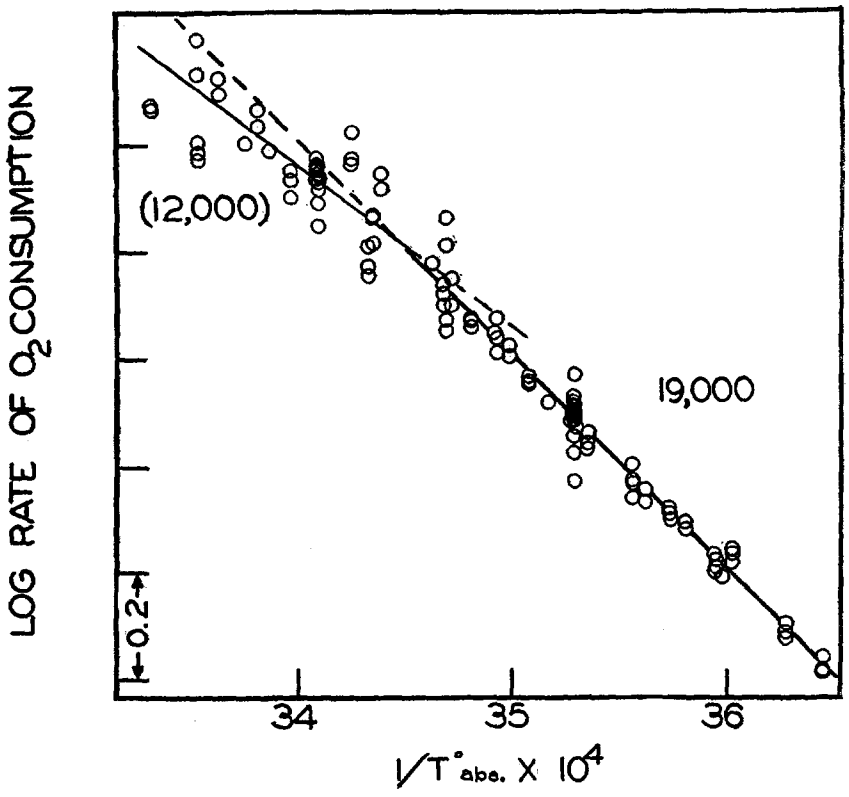

FIG. 4. All the series of experiments presented in Figs. 2 and 3 are brought together by shifting the lines to a common level at 15°. The coordinates are the same as those used in Figs. 2 and 3. The points scatter about a best fitting line giving a μ of 19,000 cal. (The probable change of slope above 15° is discussed in the text.)

not be so easily discarded. All other values lie within the range 15,800 to 19,000, on the basis of the individual series (Fig. 3). The apparent individual slopes (Figs. 2, 3) may be disturbed by the oc-

[2] Crozier *et al.*, *J. Gen. Physiol.*, 1924 to date.

currence of a break, more pronounced in some sets than in others, at about 20°. Further investigation at higher temperatures would be necessary to clear up this matter definitely. The true value of the temperature characteristic may not be far removed from that given by the line fitted in Fig. 4. If we arbitrarily weight each series according to the number of vessels and to the spacing of the temperature, multiply each by its rating and divide the sum of the figures thus obtained by the sum of the ratings we obtain $\mu = 17{,}900$. This procedure is not strictly correct as a method of averaging, but it is useful as a check. We believe that the scatter of computed values of μ is due entirely to random experimental errors in the method of measurement.

If we accept the evidence of the mass plot in Fig. 4, there is clear suggestion of a critical temperature at 15°, with change of μ; the slope for the high temperature portion is approximately $\mu = 12{,}000+$. The best fitting line in the lower temperature range (where the scatter is less) gives $\mu = 19{,}000$ — agreeing with the values found by Stier (1932–33) for O_2 utilization by yeast (3–15°), and by Lineweaver, Burk, and Horner (1931–32) for O_2 utilization by *Azotobacter*. Stier found two critical temperatures, one of which for the strain used comes at 15.7 ±0.27° and another comes at 29°. The values of μ are: 19,530 cal. below 15.7°, 12,440 cal. from that temperature to 29°, and 8,290 cal. above 29°. The points are scattered within parallel bands the width of which is greater at higher temperature ranges. It is of interest that for the same process in two different unicellular organisms the values of μ, and possibly the occurrence of a critical temperature should be so similar. If our experiments were carried to higher temperatures it might be that more definite breaks would also be found for *Chlorella*. (For a comparison of various values of μ reference is made to Stier's paper, 1932–33.)

V

Emerson found in *Chlorella* an increase to about four times the rate of respiration when dextrose was added to the medium. French, Kohn, and Tang (1934) find a rise to about twice at 18.5° and only 1.3 times at 3.5°. The question arises as to what part the respiration in absence of added dextrose plays in the O_2 consumption measured in

glucose solution, and as to the correction which perhaps should be made so that the glucose oxidation may be analyzed alone. The respiration in absence of added dextrose we may term normal. The three possibilities are (1) that all the O_2-enzyme complex is used by the glucose as fast as it is formed, cutting out completely the "normal" process; (2) that the normal process goes on as usual while the glucose oxidation proceeds independently, utilizing another source of O_2; and (3) that both the normal and glucose processes compete for the same source of active O_2 in such a way that the normal rate is decreased.

French, Kohn, and Tang (1934–35) have found that the normal respiration, if studied after removal to darkness for short periods corresponding to the times used in these experiments with added dextrose, gives a μ plot that has to be fitted with a curve or with two straight lines having quite different slopes with a critical temperature at about 11.5°; the temperature characteristics are respectively $\mu = 3{,}800$ and $\mu = 16{,}000$ above and below this critical temperature; the further analysis of this situation is considered in the succeeding paper, where it appears that the temperature characteristic for photosynthetically stored glucose alone is 19,500 (0.6–11.5°) and 3,500 (11.5–28°). If this normal process went on simultaneously with the glucose oxidation we should not expect a single straight line to fit the observations over this range. We then conclude that the μ value here found, 19,000, refers only to the oxidation of the added glucose by the cells and is not in part dependent on the "normal" metabolism evident in the absence of glucose from the medium.

SUMMARY

The temperature characteristic for the rate of O_2 consumption by *Chlorella pyrenoidosa* suspended in Knop solution containing 1 per cent glucose was studied between 1° and 27°C. with the Warburg technic. The value of μ was found to be about 19,000 $\pm$1,000 cal. There is some indication of a critical temperature at 20°C., with shift to a lower μ above this temperature. The effect of sudden changes in temperature on the rate of respiration and the variation of the latter with time at constant temperatures are discussed. It is concluded that the "normal" respiration (in absence of external glucose) does not appear in the determination of this temperature characteristic.

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