TEMPERATURE CHARACTERISTICS FOR THE METABOLISM OF CHLORELLA

III. The Catalytic Decomposition of Hydrogen Peroxide by Chlorella pyrenoidosa

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I

As some suggested mechanisms for cellular respiration postulate the formation of hydrogen peroxide as a by-product, the study of its decomposition by living cells is of some general interest. It was suggested by Warburg and Uyesugi (1923–24) that the same enzyme system is responsible for both peroxide splitting and the Blackman reaction of photosynthesis.

Yabusoe (1924–25) measured this reaction at different temperatures, using *Chlorella vulgaris*. He gives a straight line relation between rate and temperature Centigrade from 10 to 30°C., the 5° point being out of line with the others. If his data are used to test the Arrhenius equation by plotting the logarithm of the rate against the reciprocal of the absolute temperature it fits equally well except that the 30° point is too low. As disturbing effects of higher temperatures often occur, it seems more sensible to present his data in this form. The value of μ calculated from his points at 5°, 10°, and 20° is 17,500. A second experiment with points at 10° and 20°, however, gives μ = 12,000; the 30° points were disregarded in both sets for this calculation.

To compare μ for hydrogen peroxide splitting with that for other processes in *Chlorella pyrenoidosa* (Crozier, Tang, and French, 1934–35; French, Kohn, and Tang, 1934–35) it was decided to make similar measurements on this species and to confine the work to temperatures below 20°.

II

A large bottle was sterilized and half filled with 4 liters of sterile Knop's solution. It was inoculated with 150 ml. of *Chlorella* suspension grown in flasks in the usual way (Crozier, Tang, and French, 1934–35). The bottle was placed in the incubator used for the flasks, and in addition was illuminated from one side by a 500 watt projection bulb focused evenly over the whole side. Between the culture bottle and the light there was a heat screen of 4.7 cm. dilute ferrous ammonium sulfate with a little sulfuric acid. A stream of cold water through the incubator held the temperature at about 20°. After 8 days of growth with the usual mixture of 5 per cent CO_2 in air bubbled through, the culture was centrifuged and the cells were rinsed once with 35 ml. phosphate buffer of pH 6.72 and resuspended in 50 ml. of the buffer solution. This suspension was kept on ice during the day and a half required for the experiment. Each ml. contained 80 mm.^{3} of cells. A solution of H_2O_2 was made up of Merck superoxol in the same buffer.

The reaction mixture consisted of 25 ml. of the peroxide solution with which, after 15 minutes for thermal adaptation in separate flasks, was mixed 4 ml. of the cell suspension, thus making a 0.0238 M H_2O_2 solution containing 11 mm.^{3} of cells per ml. in M/15 phosphate buffer of pH 6.72. 5.00 ml. was pipetted for titration into a flask containing 100 ml. water, 15 ml. H_2SO_4 (1:2), and 5 ml. 20 per cent KI. Other samples were taken, at various time intervals, using the same pipette which had been washed and dried.

Immediately after the sample was pipetted into the titration mixture it was warmed for exactly 2 minutes on an electric hot plate, bringing it to 44°, then allowed to stand covered for 2 more minutes before the liberated iodine was titrated with $Na_2S_2O_3$ solution. Unless the solution is treated in this way the reaction is not complete, and if allowed to stand too long there is danger of loss of iodine. The first sample was not considered reliable due to gas bubbles in the mixture, so the initial concentration of H_2O_2 was determined by titrating a 5 ml. sample of H_2O_2 solution which had been mixed with 4 ml. of buffer instead of with cell suspension. Separate determinations gave 15.66 and 15.73 ml. thiosulfate to combine with the liberated iodine.

III

Experiments were made at 0.6°, 4.4°, 10.9°, 15.2°, and 20.0°. The time course of the reaction was followed at each temperature by taking samples at various intervals.

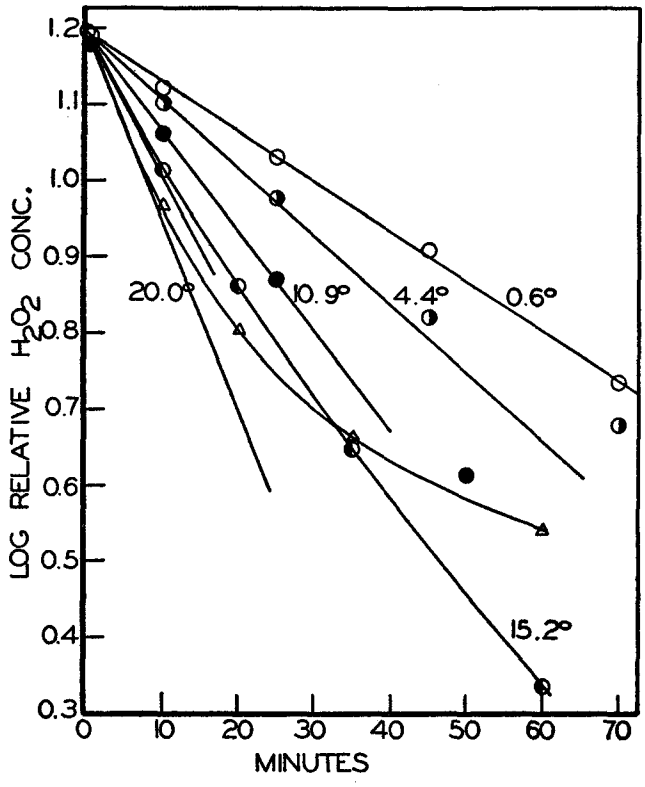

FIG. 1. The logarithms of the hydrogen peroxide concentrations (expressed in ml. of $Na_2S_2O_3$ equivalent to it) in suspensions of *Chlorella* cells, are plotted against time for experiments at five temperatures. The straight lines represent the initial slopes of the curves.

At the lowest temperature the data are accurately fitted by a first order velocity equation, from which, however, large deviations are found at higher temperatures. This equation is useful in approximating the initial rates in which we are interested. Fig. 1 shows the logarithm of the number of ml. of 0.0151 N $Na_2S_2O_3$ equivalent to the

liberated iodine plotted against time, for the various temperatures. Curves are drawn through the points and the slope of the curve at $t = 0$ is taken as a measure of initial velocity. The destruction of the active enzyme during the reaction does not influence the initial rates. If the first order equation is obeyed the curve would be straight, since the first order equation

$$K = 1/t \log \left(\frac{a}{a - x}\right)$$

may be written:

$$\log (a - x) = \log a - Kt$$

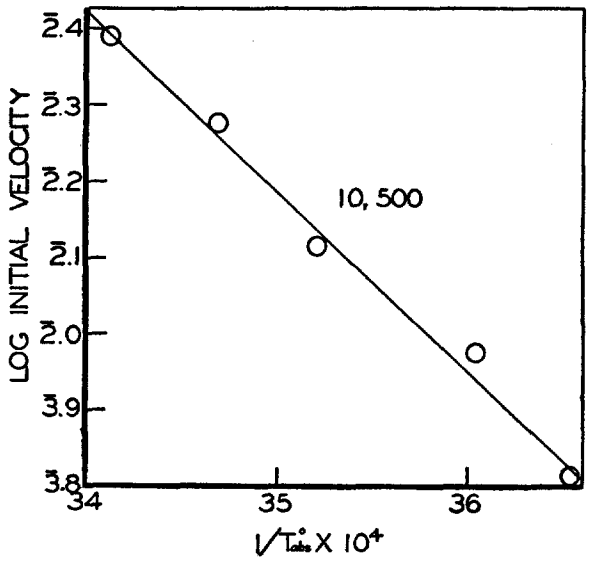

FIG. 2. The logarithms of the initial rates of disappearance of added hydrogen peroxide in suspensions of *Chlorella* are plotted against reciprocal absolute temperature. The line corresponds to a value of μ = 10,500 calories.

which is the equation of a straight line relation between log $(a–x)$ and t; a is the initial H_2O_2 concentration and $(a–x)$ is the concentration at time t; these are expressed directly in ml. of thiosulfate. The logarithms of the initial rates of H_2O_2 decomposition are plotted against reciprocal absolute temperature in Fig. 2. They are fitted by a straight line with a slope of μ = 10,500.

IV

Since the results obtained at the higher temperatures fall off considerably from first order curves, we may conclude that the substance responsible for the catalytic destruction of H_2O_2 is decomposed during the reaction. This is in accord with the usual kinetics of catalase (Williams, 1927–28; and Yamasaki, 1920) and indicates that we are dealing with an enzyme rather than with a stable surface action that might well be the cause of decomposition of H_2O_2 by intact cells. Sohngen and Smith (1924), however, find that for yeast cells the first order equation is followed at temperatures between 25–50°C. It may be that in their case the action was due to the cell wall or other stable constituents of the protoplasm. Their results give μ = 12,200 from 25–50°, substantially in agreement with this determination for *Chlorella*.

The value of μ here found, 10,500, is quite different from either the values 3500 or 19,000 found previously for respiration of *Chlorella* without added glucose and 19,000 for O_2 consumption in 1 per cent glucose solution. It is seen that various processes in the same organism need not necessarily behave the same way in relation to temperature. The interesting possibility has been suggested by Crozier that this value of μ might be changed by the presence of glucose or other substances in solution. The effect of light on the reaction is also unexplored.

SUMMARY

The decomposition of hydrogen peroxide by intact *Chlorella* cells follows a first order course at very low temperatures, but at higher temperatures gives falling first order constants. Between 0.6° and 20°C. the value of μ is 10,500 calories.

CITATIONS

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