TEMPERATURE CHARACTERISTIC FOR THE ANAEROBIC PRODUCTION OF CO₂ BY GERMINATING SEEDS OF LUPINUS ALBUS

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I

Temperature characteristics for the consumption of oxygen and for the aerobic production of CO₂ by germinating seeds of Lupinus albus were found to be different, and the suggestion was made that the mechanisms underlying the two processes are different but they may act simultaneously (Tang, 1931–32, a, b). As a means of confirmation, the differential inhibiting effect of CO was resorted to. It was demonstrated that while the rate of consumption of oxygen can be reversibly inhibited by CO, the production of CO₂ is apparently unaffected by an atmosphere of carbon monoxide (Tang, 1931–32, d). It was also pointed out that the rate of anaerobic CO₂ production is of the same order of magnitude as that in air. Although qualitative evidence was lacking regarding the metabolic identity of the CO₂ produced in the two cases, it was considered hardly plausible that the gas produced anaerobically could be quantitatively replaced by the CO₂ given off from an entirely different mechanism when air is made accessible to the respiring material. The purpose of the present paper is to attempt a direct and qualitative identification of the two processes.

The question of the identity of the processes underlying the production of CO₂ aerobically and anaerobically has attracted the attention of many experimenters. Though the evidence is on the whole in favor of identity, direct experimentation has thus far been lacking—at least to the knowledge of the writer. The conclusion has been arrived at mainly from the observations that the rates of production of CO₂ aerobically and anaerobically are affected in the same way by factors such as age, temperature, anesthetics, phosphates, and sugars.¹

¹ For a review of the subject see: Kostychev, 1927, chapter 2.

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A simple and yet direct means of identifying the two processes in an essential respect is afforded by comparing their temperature characteristics—the reverse of the logic used in the previous papers of this series (Tang, 1930-31, 1931-32, a, b, d). For a general discussion of the possible significance of temperature characteristics in biological processes, the reader is referred to the work of Crozier (1924-25, etc.).

The seeds used were in part from the same lot used in the previous experiments, and in part from a fresh lot obtained from the same source upon the exhaustion of the old supply. Preliminary experiments showed that the two lots of seeds behaved alike in relation to temperature and the effect of CO. They were treated in the same manner as described in previous accounts (e.g., Tang, 1930-31).

Modified Warburg microrespirometers designed for the study of seed metabolism (Tang, 1931-32, c) were used. The experiments were performed in darkness, except during the readings of the manometers when a very weak electric light was turned on opposite the manometers. The latter were not shaken during the experiment. The vessels, with the seeds mounted in place, were filled with N₂ by passing the gas fairly vigorously (at a pressure of 50 mm. of Brodie solution in the manometers) through them for at least 7 minutes. The N₂ was purified by bubbling it through an absorption tower containing Fieser solution, then through a solution of Pb acetate to remove any H₂S generated from the latter, dried over concentrated H₂SO₄, and finally through the respirometer vessels. When so treated the amount of O₂ in the N₂ was found to be negligible (less than 0.1 per cent when analyzed with a Haldane gas analyzer). 0.1 cc. of water was placed in each vessel to maintain a moist atmosphere.

An experiment was usually started at 18°C or 14°C, in a thermostat the temperature of which was kept constant to within 1/100 of a degree. After ½ hour of adaptation and 1 hour of respiration, during which four readings were taken, the temperature was lowered about 2°C and the process repeated until the lowest temperature of the range was reached, when the temperature was raised in a manner such that respiration data were obtained at about 1°C apart throughout the whole temperature range of the experiment. This is essentially the same procedure used in the previous studies on the temperature characteristics of

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2 The solution is made up of: 13.3 gm. of NaOH, 16 gm. of Na₂S₂O₄, 4 gm. of Na anthraquinone-β-sulfonate for each 100 cc. of H₂O. See Fieser (1924).
these seeds except that shorter ranges of temperature were employed here. This was unavoidable because, unlike that in air, the rate of production of CO₂ in N₂ was found to decrease with time, the more rapidly the higher the temperature. In Fig. 1, the course of CO₂ production for the seeds at three different temperatures is plotted against time for a period of 12 hours after ½ hour was allowed for adaptation in each case (room temperature was 24±°C.). These are representative of a large number of experiments. It is noticed that the rate is constant at 8° and almost so at 18°, which are the lower and upper limits of the temperature range used. For practical purposes, and for short durations such as 1 hour at each temperature, the rate

![Graph showing CO₂ production over time at different temperatures with coordinates.](image-url)
may be treated as being constant at temperatures lying within these limits.\textsuperscript{4} This is not the case for higher temperatures, of which the curve for 22° is fairly representative. The assumption that the rate of production of CO\textsubscript{2} between 8° and 18° during the entire period of the experiment may be treated as being constant with respect to time is justified in the following way. Table I presents two series of experiments, one of which was started at 18° and then the temperature was lowered to 16°. At each temperature ½ hour was allowed for thermal adaptation, after which readings were taken at 15 minute intervals.

\textsuperscript{3} See discussions below.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Experiments illustrating the constancy of the rates of CO\textsubscript{2} production (c.mm. CO\textsubscript{2} per seed per hour) during short time intervals at each temperature, and the absence of stimulation when temperature is changed either from a higher to a lower one, or vice versa.} & & \\
\hline
\textbf{The data are obtained from two separate series of experiments: one, in which the temperature is changed from 18° to 16°, and another, from 11° to 14°, with 30 minutes for adaptation at each temperature. Because of the small changes on the manometers, the figures are only reliable to ± 7 per cent.} & & \\
\hline
\textbf{Experiment No.} & \textbf{Successive periods of 15 min.} & 1 & 2 & 3 \\
\hline
18° & 1 & 5.8 & 7.1 & 5.6 \\
 & 2 & 6.5 & 6.4 & 4.9 \\
 & 3 & 5.8 & 7.1 & 5.6 \\
 & 4 & 7.1 & 7.1 & 4.2 \\
16° & 1 & 4.5 & 3.6 & 3.5 \\
 & 2 & 6.5 & 5.7 & 4.2 \\
 & 3 & 4.5 & 5.0 & 4.2 \\
 & 4 & 5.8 & 5.0 & 4.2 \\
11° & 1 & 3.3 & 4.3 & 4.1 \\
 & 2 & 4.6 & 6.5 & 6.9 \\
 & 3 & 2.6 & 5.0 & 4.8 \\
 & 4 & 4.6 & 5.8 & 6.2 \\
14° & 1 & 5.2 & 7.9 & 9.7 \\
 & 2 & 5.9 & 7.2 & 6.9 \\
 & 3 & 4.6 & 7.2 & 8.3 \\
 & 4 & 5.2 & 7.2 & 7.6 \\
\hline
\end{tabular}
\end{table}
for an hour. The other series was started at 11° and then raised to
14°, and the readings were taken in the same manner. The figures
are constant to within ± 7 per cent, which is the limit of accuracy of the
method for such small changes in the manometers. That temperature
alterations as practiced here and in the previous experiments did not
produce any "stimulating effect" is made evident in the following tests.
In previous studies on the temperature characteristics for the oxygen
consumption of these seeds, a set of observations were made (but not
published) in which the rates of oxygen consumption of the seeds were
observed first at 13°. After going through the usual temperature
changes, the rates were observed again at the same temperature. The
rate was 72 c.mm. per seed per hour at 13° before and after the change
for a typical experiment. During the course of the present investiga-
tion, three experiments were performed in the same manner on the
production of CO₂ in N₂, with 14° as the initial and final temperature.
The rates were 28.0, 33.0, and 20.5 c.mm. of CO₂ produced per seed
per hour at the beginning, and 29.5, 33.0, and 21.1 respectively at the
end, when the temperature of the experiment was changed successively,
about 2° at a time, from 14° to 7.5° and back to 14° with a time lapse
of about 12 hours. These preliminary experiments clearly indicate
that for the study of the rate of respiration of the seeds as a function of
temperature, under these conditions, the sudden changes of tempera-
ture and lapse of time (at lower temperatures at least) are not compli-
cating factors.

At the end of an experiment, which usually lasted for about 12 hours,
the hypocotyls of the seedlings showed little sign of elongation, though
they were somewhat larger in diameter than at the beginning. When
planted on moist sawdust, the seedlings showed healthy growth in
every case.

III

The results of two series of experiments, each consisting of five indi-
vidual observations, were brought together by factors and presented
graphically in Fig. 2. In the figure, the ordinate represents the loga-
rithms of the relative rates of CO₂ production; the abscissa, 1/T

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4 For discussion of this question see: Palladin (1899), Blanc (1916), and Harrington (1923); also Crozier and Navez (1930–31).
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× 10⁴ in Kelvin units. Where the number of circles is less than five, some of them overlap. The points fall into a band the slope of which yields 21,500 ± calories with an extreme variation of ±10 per cent.

Fig. 3 gives the results of an experiment, typical of many, where the temperature was raised from 16° to 22° and then lowered to 16°. The order in which the observations were made is indicated by the numbers near the circles. The points did not fall on any straight line in ascending the temperature scale, but in descending from 22° to 16°, they fall on a straight line the slope of which yields 22,000 ± calories. It is recalled that 19.5° was previously found to be the critical temperature both for oxygen consumption and for the production of CO₂ aerobically by these seeds, and it is likely that this is also the temperature here, but not clearly defined. Behavior of this sort in other organisms at and above the critical temperature is not unknown (e.g., Crozier and Stier, 1926–27).
Another rather unusual phenomenon, but related to the foregoing, was observed in a set of experiments of which the one given in Fig. 4 is typical. Two lines with the same slope of 23,500± can be drawn through the points; one through those observed on the downward journey of the respiring material on the temperature scale, and another upward, the absolute magnitudes for the latter being smaller. The only statement which we can make at present concerning the meaning of such effects is that phenomena of this nature have been observed in other cases (unpublished observations, Crozier and Stier). Further experimentation is needed to clear up these points.

**Fig. 3.** A typical experiment illustrating the behavior of some of the seeds at temperatures higher than 18°. The points are plotted similar to those in Fig. 2 and the order in which they were obtained is indicated by the numbers attached to the circles.

**Fig. 4.** A typical experiment illustrating the occurrence of the points on two parallel lines yielding the same slope of 23,500± calories, but different in their absolute magnitudes. The points are plotted as in Figs. 2 and 3, with the order in which they were taken indicated by numbers near the circles.
The value of $\mu$ obtained in these experiments, 21,500 (variation 20,000 to 24,000), is somewhat lower than that of 24,000± reported for the same seeds at the same temperature range, but obtained from groups of thirty seeds each, using the Ba(OH)$_2$ titration method (Tang, 1931–32, a). Another value for the same seeds obtained in five experiments performed with the Warburg technic at two temperatures (18° and 8°) only, is 23,300± calories, with an extreme variation in the values of $\mu$'s from 22,000 to 25,000 (Tang, 1931–32, b). While the mean value of 23,300± is higher than the one reported here (21,500±), the ranges of variation overlap. Curiously enough, the value of 21,500 corresponds almost exactly to that for the aerobic production of CO$_2$ for Zea mays (Tang, 1931–32, a).

That the value of $\mu$ as determined in the present experiments is on the whole slightly lower than the ones previously determined for the aerobic production of CO$_2$ by the same seeds, and that the range of variation is wider, is not at all surprising. At higher temperatures the rates of the anaerobic CO$_2$ production are only approximately constant with respect to time: actually, as has been pointed out, the rates decrease as time goes on. Because of this slight but continuous decrease in the rates, the points at the higher temperatures taken on reascending the temperature scale are lower than what they would have been if the rates were strictly constant. This is clearly revealed by comparing the points at the lower temperatures with those taken at the higher ones in Fig. 2. The former, if treated alone, tend to give a value of $\mu$ higher than 21,500, and the reverse can be said of the latter; the compromise value of 21,500 calories may certainly be considered to be slightly too low.

In view of what has been said, and also of the fact that the ranges of the individual $\mu$'s overlap, we may state that within the limits of error of the experiments, the values of $\mu$'s for the production of CO$_2$ by the germinating seeds of Lupinus albus, both aerobically and anaerobically, are found to lie within the limits of 21,000 and 24,000 calories approximately; and that the mechanisms underlying the production of CO$_2$ by these seeds aerobically and anaerobically may be considered identical in so far as concerns the relation of their rates to temperature.
The rate of anaerobic production of \( \text{CO}_2 \) by germinating seeds of *Lupinus albus* was studied as a function of temperature between 7.5\(^\circ\) and 18\(^\circ\)C. The mean value for the temperature characteristic was found to be 21,500± calories, which is slightly lower than that for the same process under aerobic conditions (23,500± calories). The values for the individual \( \mu \)'s in the two cases overlap considerably. The possible identity of the processes underlying the production of \( \text{CO}_2 \) aerobically and anaerobically is discussed.

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