ON THE RESPIRATORY QUOTIENT OF LUPINUS ALBUS AS A FUNCTION OF TEMPERATURE

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I

Previous studies on the temperature characteristics for the respiration of the germinating seeds of Lupinus albus have shown (Tang, 1930–1931, 1931–1932, a) the values of μ's for consumption of oxygen and for production of CO₂ to be different. It was inferred that the respiratory quotient of the seeds must therefore be a function of temperature. The object of the present paper is to examine this point.

II

The Warburg microrespirometer technic (Warburg, 1926) was used in this series of experiments. The customary conical vessel was replaced with a cylindrical one of special design (Tang, 1931–1932, b). It is attached to the manometer from the side instead of the top which has a removable stopper bearing a glass cross on which the seed is fastened with a sulfur-free rubber band. 0.2 cc. of 2 N KOH is placed in the bottom of the vessel and 0.3 cc. of a 2.5 N HCl in the side arm of the ordinary type. The capacity of the vessel is about 15 cc. In practice, a set of seven respirometers was used: one for thermobarometer; three, containing only KOH, were used as adaptation chambers in which the seeds were allowed to remain at the desired temperature prior to the observation. After this period of adaptation, the seeds, which are attached to the crosses on the interchangeable stoppers, are removed to the other three vessels containing KOH and HCl. In this way the CO₂ formed during the period of adaptation was not absorbed by the KOH in the respirometer. Exactly 5 minutes after the seeds were placed in the resiping chambers the stop-cocks are closed and measurements begun. At the end of 2 hours, the oxygen consumed is measured by the negative pressure on the manometer. To this is added the amount of oxygen consumed during the first 5 minutes, by extrapolation. The amount of CO₂ produced is obtained by tipping the HCl in the side arm into the vessel proper and observing the positive pressure. From this amount must be subtracted the amount of CO₂ originally present in the

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KOH solution, which is obtained by tipping the HCl in the side arm of the thermobarometer which contains amounts of solutions identical with those in the respirometer. The manometers are shaken fairly vigorously (ca. 60 complete oscillations per minute with a throw of 8 cm.) for 5 minutes after the tipping of the acid. This was found to be sufficient to liberate all the CO₂ from the alkali, without the manometric reading being altered by any gas exchange during that time.

The tipping of acid on the respiring material in order to obtain the “pre-formed” CO₂ as in the animal tissue suspended in aqueous solutions (Meyerhof, 1930; Gerard, 1927; Dickens and Simer, 1930) was deliberately avoided, since preliminary experiments showed that not only was there no CO₂ given off when the seeds were so treated, but that the manometer actually showed a slight negative pressure which is not prolonged for more than a few minutes and is decidedly different from the phenomenon observed by Amberson, Armstrong, and Root (1931).

The pressure changes, multiplied by the suitable vessel constants, give the volumes of gas exchanged. The constants are given by the equation:

\[
K = \frac{V_s \times \frac{273}{T} + V_f \times \alpha}{10,000}
\]

in which \(V_s\) is the volume of the vessel minus those of the respiring material and liquid \((V_f)\). \(T\) is the temperature of the experiment in Kelvin units, \(\alpha\) the Bunsen coefficient of solubility of O₂ or CO₂ in water at \(T\), and 10,000 is the atmospheric pressure in terms of Brodie solution (Warburg, 1926). The volumes of the vessels were calibrated by any convenient method (Oppenheimer, 1928; Warburg, 1926), and the volume of the seed was obtained by immersing it in water in a graduated centrifuge tube. Since the solubility of gases in water at different pH is not exactly known, \(V_f\) was made so small that the term \(V_f \times \alpha\) vanished even if the Bunsen coefficient of solubility were taken, rendering incidentally the \(K\) for both CO₂ and O₂ identical.

After the necessary data were obtained, the temperature of the thermostat, which is maintained constant to 0.01°, is changed. The seeds are placed in the three original chambers for thermal adaptation while the three others, together with the thermobarometer, are removed for washing, cleaning, and refilling with new solutions. They are then returned to the thermostat, and after thermal equilibrium is attained, are used for the next experiment.

The seeds are the same as those used in the previous work (Tang, 1930–1931) and are treated in exactly the same way. The experiments were performed in darkness; the manometers were not shaken during the actual experiment.

III

For production of CO₂ by *Lupinus albus*, the value of \(\mu\) was found to be 24,000± calories, and for consumption of oxygen 16,600±
calories, below the critical temperature of about 19.5°. According to the Arrhenius equation, for CO₂ production we have:

\[
\log K_{2}^{\text{CO}_2} - \log K_{1}^{\text{CO}_2} = \frac{\mu_{\text{CO}_2}}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \tag{1}
\]

Similarly, for oxygen consumption,

\[
\log K_{2}^{\text{O}_2} - \log K_{1}^{\text{O}_2} = \frac{\mu_{\text{O}_2}}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \tag{2}
\]

Subtracting (2) from (1), we have

\[
\log \frac{K_{2}^{\text{CO}_2}}{K_{1}^{\text{CO}_2}} - \log \frac{K_{2}^{\text{O}_2}}{K_{1}^{\text{O}_2}} = \frac{\mu_{\text{CO}_2} - \mu_{\text{O}_2}}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \tag{3}
\]

If \( K \) is measured in terms of volume (or units proportional thereto) per unit time, (3) becomes

\[
\log RQ_{T_2} - \log RQ_{T_1} = \frac{\mu_{\text{CO}_2} - \mu_{\text{O}_2}}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)
\]

Rearranging,

\[
\frac{RQ_{T_2}}{RQ_{T_1}} = \frac{\mu_{\text{CO}_2} - \mu_{\text{O}_2}}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right) \tag{4}
\]

This is the general expression relating the respiratory quotient (\( RQ \)) to temperature and to the difference in the values of \( \mu \)'s. It will be noted that it is the difference of the \( \mu \)'s that is concerned here and not their absolute values. Substituting for \( \mu_{\text{CO}_2} \) and \( \mu_{\text{O}_2} \), the values 24,000 and 16,600 respectively, taking \( R = 2 \), \( T_2 \) as 291, and \( T_1 \) as 281, and converting to Briggsian logarithms,

\[
\log_{10} \frac{RQ_{291}}{RQ_{281}} = 0.196 \tag{5}
\]

and

\[
\frac{RQ_{291}}{RQ_{281}} = 1.57 \tag{6}
\]

Allowing a ±5 per cent variation in the values of \( \mu \)'s, as might be expected from the fluctuations of \( Q_{\text{O}_2} \) and \( Q_{\text{CO}_2} \) at constant tempera-
ture (cf. Tang, 1930–31, a, b), and taking the differences of the extreme values, the ratio in (6) should fluctuate between the limits 1.39 and 1.78. Table I gives the data obtained experimentally, using seeds from the same lot, with the same germination treatment as previously described (Tang, 1931–1932, a).

The mean of the five ratios of R.Q.₈₀/R.Q.₈₄ is 1.51 with extreme values of 1.35 and 1.71, which are in good agreement with those demanded in equation (6). The values of μ's for both CO₂ production and oxygen consumption agree satisfactorily with those obtained previously. These values were obtained regardless of whether the seeds were subjected to the higher temperature first and then to the lower or vice versa, provided adequate time was given for thermal adaptations which in this case takes at least 2 hours. Table I shows also the variability of $Q_{CO₂}, Q_{O₂}$, as well as of the R.Q.'s, among the individual seeds. The uniformity of the Q's is not materially improved when expressed in terms of fresh or of dry weights, or in terms of volume or surface of seed.

IV

The results of this series of experiments, together with those already published (Tang, 1931–1932, a, b), clearly bear out two interesting

### Table I

Respiratory Quotients of Germinating Seeds of Lupinus albus at 18° and 8°C., and the Calculated Value of μ

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>18°</td>
<td>8°</td>
<td>18°</td>
<td>8°</td>
<td>18°</td>
<td>8°</td>
</tr>
<tr>
<td>$Q_{CO₂}$ c. mm./seed/hr</td>
<td>78.0</td>
<td>20.5</td>
<td>76.0</td>
<td>18.0</td>
<td>61.0</td>
<td>15.0</td>
</tr>
<tr>
<td>$Q_{O₂}$ c. mm./seed/hr</td>
<td>88.0</td>
<td>31.0</td>
<td>93.0</td>
<td>32.5</td>
<td>77.0</td>
<td>27.0</td>
</tr>
<tr>
<td>R.Q.</td>
<td>0.89</td>
<td>0.66</td>
<td>0.82</td>
<td>0.55</td>
<td>0.79</td>
<td>0.56</td>
</tr>
<tr>
<td>R.Q. 18°/R.Q. 8°</td>
<td>1.35</td>
<td>1.49</td>
<td>1.41</td>
<td>1.71</td>
<td>1.59</td>
<td>1.51</td>
</tr>
<tr>
<td>$μ_{CO₂}$</td>
<td>22,000</td>
<td>23,500</td>
<td>23,000</td>
<td>25,500</td>
<td>23,000</td>
<td>23,300</td>
</tr>
<tr>
<td>$μ_{O₂}$</td>
<td>17,100</td>
<td>17,200</td>
<td>17,200</td>
<td>16,600</td>
<td>15,100</td>
<td>16,400</td>
</tr>
</tbody>
</table>
points; viz., the temperature characteristic for the production of CO₂ by *Lupinus albus* is different from that for the consumption of O₂, and the respiratory quotient accordingly changes with temperature.

That the difference in the temperature characteristics for the two phases of respiration is neither an accidental occurrence nor a technical artefact is made evident by the fact that such a difference was not observed in *Zea* (Tang, 1931–1932, b), and also by the fact that it occurs in *Lupinus albus* regardless of the technic used.

Granting that the difference in the temperature characteristics is a real one, we are confronted by three possible interpretations of this phenomenon. The first and obvious one is that suggested in an earlier paper (Tang, 1931–1932, b), namely that the mechanisms governing the production of CO₂ and the consumption of oxygen may be different although acting simultaneously. This is not at all improbable, especially in the light of the modern conception of the relationship between fermentation and respiration (cf. Kostychev, 1927, Chapter 3). Indeed, recent investigations by Blackman and Parija (1928), and those of Chivellard et al. (1931), with apples, potatoes, mosses, etc., at different oxygen tensions come to almost the same conclusion. However, these authors are of the opinion that at higher oxygen tensions respiration (oxidation) takes place exclusively.¹ Although their conclusions may be entirely sound, one hesitates to give much weight to experiments where such bulky materials as apples and potatoes are used, and where the experiments are carried out over too long a time.

A second interpretation of the difference in the temperature characteristics might be offered by assuming a difference in the respiratory activities of the cotyledons and of the plantule, as in the case of *Ricinhus* seeds discussed by Murlin (1932) among others. If this were true, it is hardly conceivable that any regularity should occur in treating the respiration of the whole seed as a function of temperature, and it is certainly inconceivable that the ratio of the respiratory quotients should come out exactly as predicted by the use of equation (4) which is derived from the assumption that the difference in the temperature characteristics is a real one, due to specific differences in

¹ Blackman and Parija are of the opinion that glycolysis is the common process governing both oxidation and fermentation.
the mechanisms underlying the two processes. That the individual ratios in Table I deviate rather markedly from the average of 1.51 as pointed out above, is not a strong objection in this case. It must be remembered that in (6) the value of 1.57 is derived from a logarithmic value of 0.196 of (5). A small deviation in the latter caused by taking slightly different values of the \( \mu \)'s, reflects a considerable variation in the former, as was anticipated. Moreover, the r.q.'s are computed from observations at two temperatures only, and the temperature characteristics must be expected to show some variation when so obtained, as earlier pointed out, and the variations observed fall within expected limits.

A third explanation may be attempted by supposing an inadequacy of diffusion of oxygen into the seeds. If this were true, there would be created at least two zones in the seed—an outer one of oxidation, an inner one of anaerobic cleavage; the depth of the former depending on the rate of diffusion of oxygen into the seed. Had the coefficient of diffusion of oxygen into plant materials been known, as in the case of animal tissues (cf. Krogh, 1919; Tang and Gerard, 1932), the problem could have been dealt with by a simple calculation (cf. Warburg, 1926; Hill, 1928–1929). Unfortunately such data are unavailable, and we have to dismiss the question with the rather unsatisfactory statement that there was no significant rise in the rate of oxygen consumption of our seeds when they were subjected to an oxygen atmosphere as compared with their rate in air, indicating that the diffusion of oxygen is presumably not a limiting factor in this case. Even if diffusion were the limiting factor, the rate of production of CO\(_2\) from the inner zone must be considerably higher than that of the outer (where both CO\(_2\) production and O\(_2\) consumption occur) in order to give an apparent and dominant temperature characteristic. Then, in all probability, the total amount of CO\(_2\) produced per unit time by the seed as a whole will be in excess of the oxygen consumed. But in reality, the respiratory quotient, even at the higher temperature (18\(^\circ\)), is never equal to unity, and is about 0.80.

Thus in view of absence of direct evidence against it, and also because of the close agreement of the ratios of the respiratory quotients at 18\(^\circ\) and 8\(^\circ\) with that demanded by equation (4), we may work on the hypothesis that in the seeds of Lupinus albus at least, the produc-
tion of CO₂ and the consumption of oxygen may be simultaneous and yet governed by two different mechanisms.

The second point of interest which can be deduced from the measurements, and is in reality a corollary to the preceding paragraph, is the change of respiratory quotient with temperature. In going over the literature on plant respiration, we find the question of the change of respiratory quotient with temperature an old one. While it is not the aim of this paper to give a comprehensive review of the literature, a brief summary may be made. Bonnier and Mangin (1884), Dehénarin and Moisson (1874), and Aubert (1892), maintained that temperature changes do not affect the respiratory quotient; Pouriewitch (1905) later questioned their conclusions and showed in all the plant materials which he studied, including the seedlings of Lupinus albus, a change of the quotient with temperature. Recently Harrington (1923) also found a change in the respiratory quotient with temperature in his study of the respiration of apple seeds. These authors, however, attribute the change, if it does occur, to the change in the nature of the food materials being consumed. The experiments reported here give a possible explanation of the phenomenon other than that offered by Pouriewitch and Harrington (cf. also Kostychev, 1927, Chapter 1). If the mechanisms governing the production of CO₂ and the consumption of oxygen are different, and are affected differently by temperature, a change of the respiratory quotient with temperature is the necessary consequence. In such cases we ought to be able to predict the change not only qualitatively, but also quantitatively. This we have done. These two interpretations of the change of the respiratory quotient with temperature are not necessarily mutually exclusive. It is perfectly conceivable that in certain cases the kinds of food material may be the cause for the change, while in others the nature of the reaction mechanisms. The two may or may not be interdependent.

The writer is aware that the respiratory quotient has been thoroughly investigated in animals, especially in man, and he does not doubt the usefulness of the respiratory quotient in clinical investigations where it is treated solely as a function of food materials consumed—provided it be determined with due care (cf. Richardson, 1929; and Cathcart and Markowitz, 1927). Even in human respiration, the
Respiratory quotient of Lupinus albus

Quotient does change with the temperature of the body (Landis et al., 1926); but there it is due principally to the change in the pH of the blood and to the difference in the rate of pumping out of the gases from the lung. The phenomenon is thus rendered complicated for analyses of the kind employed here.

Summary

The temperature characteristics for the oxygen consumption and CO₂-production of the germinating seeds of Lupinus albus were previously found to be different. It was predicted qualitatively that the respiratory quotient of the seed should be a function of temperature. A quantitative treatment is presented here, relating the change of the respiratory quotient with temperature and the temperature characteristics. Experimental results agree satisfactorily with the calculated value.

Citations

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