THE EFFECT OF ACCUMULATED CARBON DIOXIDE ON PLANT RESPIRATION*

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In a study of the biochemical basis of winter hardiness in apple trees, the writers have had occasion to measure the relative rates of respiration of tender and hardy varieties. In the first series of measurements (4) continuous aspiration was employed; that is, during the period of measurement CO₂-free air was continuously passed through the chamber containing the twigs.

Because of certain mechanical difficulties in this method over long periods, discontinuous aspiration was then tried; that is, the CO₂ was allowed to accumulate in the chamber and was measured at the end of the period. It became apparent that an entirely different picture of respiratory activity was obtained by this procedure. Therefore, a more detailed study of the phenomenon was undertaken, the results of which are presented in this paper.

As long ago as 1881 MüNTZ (16) reported that grain emits many times as much CO₂ when it has access to fresh air as when it is confined in a container. Mangin (15) in 1896 pointed out that the respiration of germinating seeds is decreased by the presence of 5 per cent. of CO₂, and that the respiratory quotient becomes greater. Czapek, in his excellent review of plant respiration (12), does not discuss this particular phase of it. Many studies on respiration have been conducted by both methods. The well-known OSTERHOUT method (19) is of the continuous type. Bailey and Gurjar (1, 3) used the discontinuous method on grains, conceding that it indicated a rate that continually decreased with time. SpoeHR and McGee (23) described quite definitely the effect of changing plants from one concentration of CO₂ to another, which in effect is really the procedure in the discontinuous method. Olney (18) used the latter method on bananas, while Bergman (6) employed the continuous on cranberry plants. The present writers used the continuous method in the work on apple twigs reported preliminarily (4). Thomas (25) has found that concentrations of CO₂ above 12 per cent. if the air surrounding apples tend to increase the production of ethyl alcohol and of acetaldehyde; in other words, to change the respiration to a zymasic type.

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In the light of the facts briefly reviewed above, it is evident that the accumulation of CO₂ in the atmosphere surrounding plant tissue has an appreciable effect on the respiration of that tissue.

Experiments with apple twigs

In the work reported here the twigs were gathered during the first two weeks in March, 1926. Only the one-year-old wood was used. The cut ends were paraffined. The twigs were exposed to room temperature for only a few minutes. From 150 to 200 gm. of the twigs were packed into glass tubes about 5 cm. in diameter and about 45 cm. long, and the tubes placed in the thermostat immediately. The temperature was 0° C. in these

![Graph of apple twig respiration at 0° C.](image)

Fig. 1. Respiration of apple twigs at 0° C. The dotted line represents the rate during a period when the CO₂ is allowed to accumulate; the solid line represents a 20-minute period of continuous removal of CO₂. A, B, C, and D are different lots of twigs.
experiments. The thermostat, controls, air washing and circulating devices, and absorption towers were the same as described elsewhere (5).

The data are presented in figure 1. The vertical axis represents the rate of CO₂ production, expressed as mg. of CO₂ per hour per 100 gm. of twigs. The horizontal axis represents successive periods of time. The dotted lines stand for periods during which the CO₂ was allowed to accumulate, followed by a 20-minute period of aspiration to remove the CO₂. The lengths of these periods in hours is indicated by the number at the end of the dotted line. The solid line represents a 30-minute period, 20 minutes of which were consumed in aspiration, and 10 minutes in changing apparatus in preparation for the next period of aspiration. These half-hour periods thus represent periods of practically continuous aspiration. The time required for complete removal of CO₂ in the chamber, was, of course, carefully determined, and found to be about 12 minutes; 20 minutes were then adopted for safety. The object of this schedule was to measure the rate of CO₂ emission during varying periods by the discontinuous principle, followed by its measurement with continuous aspiration. Sections A to D represent different lots of twigs.

It will be seen that each dotted line or each series of them, is followed by a series of solid lines, each succeeding one of the latter being shorter. This means that a period in which the CO₂ is allowed to accumulate in the chamber is followed immediately by a much higher rate of respiration. The rate gradually subsides, but in the one case in lot B where a constant rate was attained, it was after about 35 hours.

There is some evidence that the magnitude of this phenomenon is proportional to the amount of CO₂ that has accumulated in the chamber, or in other words, the length of time of this accumulation. In lot C it is slightly evident after a 2-hour period. It is most pronounced following the 164.5 hour run in B and the 64.1 hour run in C. Seven periods of moderate length in B are as effective as two periods of much greater length in D.

It is quite evident that from these data, it would be impossible to say what is the normal rate of respiration of these twigs. Continuous removal of the CO₂ is imperative.

Casual study of the data for the accumulation periods indicates that in general the rate for the period is inversely proportional to the length of the period; in other words, that the rate continuously decreases with time. In order to bring out this relation more clearly, the amount of CO₂ for each period was plotted against the length of the period. The resultant graph is shown in figure 2.

A curve was fitted to these points. This curve corresponds to a logarithmic one expressed by the formula:

\[
\frac{\text{CO}_2}{\log t - 0.566} = k
\]
Fig. 2. Respiration of apple twigs at 0° C. as determined by the accumulation method. The symbols along the solid line represent different lots of twigs, with the CO₂ plotted against time in hours. The dotted line represents CO₂ plotted against log of time, using points on the solid line.
The solid line is CO₂ against \( t \) in hours, while the dotted line is CO₂ against log \( t \). Table I gives the values of \( k \) for certain values of \( t \).

**TABLE I**

VALUES OF \( k \) FOR THE RESPIRATION OF APPLE TWIGS ACCORDING TO THE CURVE IN FIGURE 2

<table>
<thead>
<tr>
<th>( t ) hours</th>
<th>CO₂ ( mg. )</th>
<th>( k ) log ( t - 0.566 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10.0</td>
<td>75.2</td>
</tr>
<tr>
<td>10</td>
<td>17.7</td>
<td>40.8</td>
</tr>
<tr>
<td>20</td>
<td>28.0</td>
<td>38.1</td>
</tr>
<tr>
<td>30</td>
<td>34.6</td>
<td>38.0</td>
</tr>
<tr>
<td>40</td>
<td>39.3</td>
<td>37.9</td>
</tr>
<tr>
<td>50</td>
<td>42.7</td>
<td>37.7</td>
</tr>
<tr>
<td>70</td>
<td>48.2</td>
<td>37.7</td>
</tr>
<tr>
<td>90</td>
<td>52.2</td>
<td>37.8</td>
</tr>
<tr>
<td>100</td>
<td>53.9</td>
<td>37.6</td>
</tr>
<tr>
<td>130</td>
<td>58.3</td>
<td>37.7</td>
</tr>
<tr>
<td>170</td>
<td>62.7</td>
<td>37.7</td>
</tr>
</tbody>
</table>

The curve is in satisfactory agreement with the experimental values. The log-time curve is a straight line beyond the 30- or 40-hour period. During the shorter periods it is not a straight line because the respiration values are affected by the high temporary rates at the beginning of each period, in accordance with the data of figure 1 previously discussed. If the short period measurements had followed in all cases periods of continuous aspiration, during which the rate of CO₂ emission had attained a constant value, these short period measurements would no doubt have conformed much more closely to the equation.

This fact emphasizes the necessity, in plant respiration work, of taking cognizance of the history of the material during the period immediately preceding that of the measurement.

**Experiments with potato tubers**

Small potato tubers which would just enter the respiration tube were used in the same type of experiments. The tube was kept on the laboratory table at room temperature without special control. Any change in temperature during the continuous phase of aspiration was guarded against, and this is the only place that small changes in temperature could change the present results.
The results with two lots of tubers are shown in figure 3. The effect of accumulated CO₂ on the subsequent rate of its production is practically the same as in the case of the twigs.

Experiments with wheat

Since the results of Bailey and Gurjar (3) indicate that the respiration of wheat grain may be affected by accumulated CO₂ in a manner similar to apple twigs, a series of experiments was run to determine this effect. Sound Marquis wheat was tempered to contain about 16 per cent. moisture. Respiration tubes like the ones used for twigs were filled with the wheat, about
500 gm. being required. The tubes were placed in a water thermostat at 40° C. This relatively high temperature was used because of the much slower rate of CO₂ production of this material in comparison with twigs.

The results are presented in figures 3, 4 and 5. In these runs the 20

![Diagram](image.png)

Fig. 4. Respiration of wheat at 40° C. See fig. 1 for description of conventions.

minutes of aspiration was followed immediately by a second aspiration. Therefore the solid lines represent 20-minute periods instead of 30. Practically the same picture is obtained here as in the case of the twigs and of the tubers. Each period of accumulation is followed immediately by an increased rate of CO₂ production. The rate gradually subsides, probably becoming constant after two or three hours, although this was not attained in any of these runs.
A possible explanation of the CO₂ effect

It has been shown that three types of plant tissue, twigs, tubers and wheat grain, exhibit the same reaction to the accumulation of CO₂ in the surrounding atmosphere. For the sake of clearness, this reaction may be described again: When the CO₂ is allowed to accumulate, the rate of production of the CO₂ diminishes in a logarithmic ratio. In the ease of twigs, at least, the amount of CO₂ produced is proportional to the logarithm of time. When accumulated CO₂ is removed, the rate of its production immediately assumes a far higher value; and the magnitude of this increased value is possibly proportional to the amount of CO₂ previously accumulated. It is a matter of several hours before the rate attains a constant value.

This is in strict accord with the conclusions of Spoehr and McGee (23): "When the CO₂-content of the air surrounding a leaf is changed from a lower to a higher concentration, the leaf shows a reduced rate of CO₂ emission for a period following the change, then increases, and finally again attains about the same rate as before the change in CO₂-content was made. Conversely, when the CO₂ content of the air surrounding a leaf is changed from a higher to a lower concentration, the leaf shows a primary increased rate of CO₂-emission and subsequent decrease to the original rate."

Such being the facts, we are of course interested in attempting an explanation. One explanation is that we are observing merely an equilibrium between the CO₂ in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO₂ in the latter is removed but slowly when aspiration is commenced.

Another possible explanation was suggested by Dr. R. A. Gortner. It is that the accumulation of CO₂ in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point, and hence increases its permeability, which is responsible (perhaps through increased enzyme activity) for an actual increased rate of CO₂ production. The increase would probably be merely potential as long as there was a high content of CO₂ in the air surrounding the tissues, but would become actual as soon as aspiration was commenced.

It will be necessary to review the existing evidence in favor of such a proposition before presenting the experiments designed to demonstrate it.

That the membranes would be more permeable if their proteins were nearer the isoelectric point is possibly illustrated by the experiments of Hitchcock (13). We can best quote from him directly: "The permeability of gelatin-coated collodion membranes, as measured by the flow of water or of dilute solutions through the membranes, has been found to vary with the pH of the solutions. The permeability is greatest near the isoelectric point of the protein; with increasing concentration of either acid or alkali it
decreases, passes through a minimum, and then increases. These variations with pH are qualitatively in accord with the assumption that they are due to swelling of the gelatin in the pores of the membrane, the effects of pH being similar to those observed by Lœb on the swelling of gelatin granules. Indications have been found of a similar variable permeability in the case of membranes coated with egg albumin, edestin, serum euglobulin, and serum albumin.”

That the acidity of the tissue fluids is increased by the accumulation of CO₂ is well known, and does not need a specific illustration. That such an increase in acidity would bring the proteins nearer to their isoelectric points is, however, not so easy to argue from existing data. The isoelectric point of but few plant proteins has been determined. Chibnall (8, 9, 10, 11) has isolated glutelins from the leaves of spinach, corn, alfalfa and other plants, and has found them to have very similar properties, with an isoelectric zone of pH 4.0 to 5.0. In all cases the reaction of the expressed sap was alkaline with respect to the isoelectric point of the protein. The gliadin of the wheat berry has an isoelectric point of 6.6 according to Eto, and 5.76 according to Hoffman and Gortner (2, p. 245); that of the glutenin is 6.8 to 7.0 (2, p. 250); and that of the leucosin is 4.6 (2, p. 253). Robbins (20, 21, 22) has determined what he believes to be the isoelectric point for tissues, and has obtained the following values: potato tuber, 6.0 to 6.4; mycelium of Rhizopus nigricans, 5.0; that of Fusarium lycopersici, 5.5; that of F. oxysporum, 4.9; that of Gibberella saubinetii, 6.2; soy bean root tips, 6.2 to 6.4.

The most careful measurements of the pH of cell sap are those by Needham and Needham (17) and by Chambers and Pollack (7), in which experiments indicator dyes are introduced into the cell by micrurgical technique. The former obtained values of 6.6 for various marine ova, and the latter 6.6 to 6.8 for starfish eggs. When the cell is injured, as by a tear, the pH rapidly diminishes to pH 5.4 to 5.6. If the latter phenomenon holds also for plant cells, the conclusion is that the host of measurements of the pH of expressed sap indicate a higher acidity than actually obtains in the normal tissue. Most of such expressed saps show a pH of 5.0 to 7.0.

Taking a general survey of the data reviewed above, it is fair to conclude that there is some indication that plant sap is alkaline with respect to the isoelectric point of its proteins. Chibnall has the only direct evidence of this. If this relation be assumed, the conclusions would follow that CO₂ could bring the reaction nearer to the isoelectric point of the proteins, that this would increase the permeability of the protoplasm, and that more rapid respiration could result.

A method used in the attempt to obtain more or less direct evidence of this chain of events was to introduce HCl gas, for short periods, into the
respiration chamber containing wheat and to observe whether this increased the rate of emission of CO₂. This has been done, with fairly positive results in favor of the above hypothesis.

The air entering the chamber was first bubbled through a solution of HCl for 30 minutes in one run, and for 1.5 hours in the other. The chamber then remained for one hour without aspiration. Then the aspiration was continued and the CO₂ determined. The titration procedure was modified
so as to allow for any HCl that might accompany the CO₂. HNO₃ was used for titrating instead of the usual HCl; Ba(NO₃)₂ was used to precipitate the carbonates instead of BaCl₂; and at the end of the acid titration the chlorides were titrated with silver nitrate. Only a few mg. of HCl reached the absorption tower.

The results of the two experiments are given in figure 6. In the trial run shown in the upper portion of the graph, aspiration was continued for one hour so as to establish the rate of CO₂ production. Then the air was bubbled through concentrated HCl for 30 minutes. After standing for one hour, the accumulated CO₂ was measured, and is represented by the dotted line in C. Following this there is seen to be the usual increase in rate, followed by a decrease to a level which is somewhat lower than the rate in A. Another one-hour period of standing (D) was followed by another but slighter increase.

In the second run, in the lower half of figure 5, a control period without HCl was measured first. This involved a cycle of three periods (A, B, C). Following this was the treatment with HCl as indicated in the chart, and the measurement of the subsequent rate of CO₂ production. Period C, following the control, is characterized by an initial rate of 3.5 mg. Period F, following the HCl treatment, is characterized by an initial rate of 5.4 mg.

The writers are convinced that the HCl treatment has duplicated the effects of accumulated CO₂. To be sure, the wheat was sooner or later injured by the treatment, but it was not visible during the course of the runs, and in any event the increase in CO₂ production is a fact.

Another line of evidence that disfavors the idea of mere solution of CO₂ in the tissues is the size of the three tissues used. It is reasonable to expect that the thicker the tissue, the slower will be the rate of diffusion of the dissolved CO₂ into the surrounding air. In the three tissues used the time required to attain a constant value should decrease in the order, wheat, twigs, tubers. There is no evidence of this differential rate in the charts.

The writers do not believe that the exhaustion of oxygen from the chamber is a deciding factor. In the case of the twigs and the tubers, the oxygen was never exhausted; in the case of the wheat it was exhausted only during a few long runs. Furthermore Karlson (14) states: "The effects of ether, benzene, and alcohol on the aerobic and anaerobic production of CO₂ by wheat (seedlings) are closely similar. This would seem to indicate that the fundamental processes or the master reactions on which they depend are similar."

On the basis of these experimental findings and of the suggestive evidence of the pH values found in the literature, the writers are led to adopt
the explanation here outlined for the effect of accumulated CO₂ on the rate of respiration of plant tissues.

Conclusions

The respiration of apple twigs at 0° C., of potato tubers at about 22° C., and of wheat grain at 40° C. has been studied from the standpoint of the effect of allowing the CO₂ to accumulate in the respiration chamber. Under such conditions the rate of CO₂ production continuously decreases with time. After the first 30 or 40 hours the relation is expressed by the formula

$$\frac{\text{CO}_2}{\log t - 0.566} = k.$$  

During the first 30 or 40 hours the rate is affected by a phenomenon which can be described as follows: When aspiration of the atmosphere surrounding the tissue is commenced, after a period of accumulation of CO₂, the rate of respiration immediately assumes a far higher value than it had during the accumulation period. The magnitude of this value is possibly proportional to the amount of CO₂ previously accumulated. It is a matter of several hours before the rate attains a constant value.

One explanation of this phenomenon is that we are merely observing an equilibrium between the CO₂ in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO₂ in the latter is removed but slowly when aspiration is commenced.

Another possible explanation is that the accumulation of CO₂ in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point and hence increases the permeability of the protoplasm; and that this is responsible for an actual increase in rate of CO₂ production. The evidence in the literature on the pH of cell sap and on the isoelectric points of plant proteins bears out this view to a certain extent. Direct evidence in its favor was secured by passing HCl gas into a respiration chamber containing wheat grain. A duplicate of the CO₂ effect was obtained.

Although admitting that the proof for the latter explanation is still far from complete, the writers nevertheless subscribe to it, and offer it for the criticism of others.

The conclusion should be emphasized that the investigator should take cognizance of the CO₂ effect in deciding which procedure, the continuous or the discontinuous, to adopt for the work in hand. Under some circumstances, of course, as in a study of grain and fruit in storage, more useful knowledge might be secured by the accumulation method.

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LITERATURE CITED


