CHEMICAL NATURE OF THE OXYGEN-TRANSFERRING FERMENT OF RESPIRATION IN PLANTS

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(WITH THREE FIGURES)

Introduction

CHEMICAL NATURE OF OXIDATION FERMENTS IN ANIMAL CELLS AND BACTERIA

The effect of carbon monoxide on the respiration of animal cells and aerobic bacteria (10), and on anaerobic butyric acid fermentation (6), has shown that the ferments which act as catalysts in these energy-yielding cellular processes are compounds of heavy metals: for carbon monoxide reacts at low temperatures only with heavy metals, and respiration as well as anaerobic butyric acid fermentation is stopped at low temperatures by carbon monoxide (6, 10). It has been further shown that these catalysts of respiration and of the anaerobic butyric acid fermentation are compounds of iron: for, of all the combinations of carbon monoxide with heavy metals only those with iron are dissociated by visible light, and the cell which in the presence of carbon monoxide stops respiring or forming butyric acid, recommences its metabolic reactions when irradiated by light (7, 10, 11).

Two iron ferments, the oxygen-transferring ferment of respiration and the anaerobic-splitting ferment, are apparently in too small a concentration and are too unstable to be isolated from the living cells by the usual methods of analytical chemistry. By combining them with carbon monoxide and by measuring the various degrees of dissociation of the ferment-CO combinations (by the increase of the respiration or of the anaerobic-splitting metabolism when irradiated by different wave lengths of visible light) an indirect method was found for examining the ferments and determining their absorption spectra, their "color," within the structure of the living cell, as it were, without separating them chemically from the other cellular substances. After the absorption spectrum of the ferment-CO combination had once been found, it was possible to compare it with the absorption spectra of substances which are accessible and can be analyzed by the ordinary methods of chemistry. In this way WARBURG (12) discovered that the constitution of the oxygen-transferring ferment of respiration in animal body cells and aerobic bacteria was phaeohaemin.

Neither alcohol fermentation nor lactic acid fermentation can be affected by carbon monoxide (11), and according to WARBURG and CHRISTIAN (13) "there are also oxidation processes which are insensitive to carbon monoxide as in Chlorella, yeast press juice and anaerobic bacteria." From these anaerobic bacteria it was possible to separate a catalytically active substance,
an iron-free oxidation ferment, the so-called yellow ferment of Warburg (13, 14). So, at present we know the chemical constitution of two respiration ferments: the oxygen-transferring ferment, phaeohaemin, in animal cells and aerobic bacteria; and an oxygen-transferring ferment, the yellow ferment, in anaerobic bacteria.

**Previous data on effect of carbon monoxide upon respiration of plant cells**

The influence of carbon monoxide upon the respiration of plant cells was studied by Emerson (3), and Padoa and Vita (8). Emerson’s experimental material was the alga Chlorella. As the result of his investigations Emerson states that carbon monoxide has no effect upon the respiration of plants, which are autotrophic (i.e., which obtain their energy by the “self-nourishment” of photosynthesis). Only after he succeeded in changing this original metabolism of plants into a heterotrophic metabolism, that is to say into a “parasitic” metabolism, by suspending the cells in a special sugar-containing nutrient medium, did the alga become sensitive to carbon monoxide. Under these conditions Emerson found, in spite of a rather low partial pressure of oxygen and a high partial pressure of carbon monoxide (2.5 per cent. O₂, 97.5 per cent. CO; ratio CO/O₂ = 39), only a small inhibition of respiration (15 to 25 per cent.). The experimental material of Padoa and Vita (8) was Plantago major, Lemna minor, and Elodea canadensis. These authors report that the respiration of plants is not inhibited by CO, but that, on the contrary, CO increases their respiration.

**Experimentation**

The experiments discussed in this paper endeavor to answer the following questions: What is the chemical nature of the oxygen-transferring ferment of respiration in plants? Can this ferment be identified with one of the two oxidation ferments already known: the iron-free yellow ferment which is insensitive to carbon monoxide, or phaeohaemin which is sensitive to carbon monoxide? The writer has determined the effect of carbon monoxide on the respiration of the stamens of plums, stamens and pistils of daffodils, Spanish moss, green pine needles, and green leaves of tobacco, plum, and oleander plants. In all these plant cells the writer found that CO produces a strong reversible inhibition of respiration. For instance, in an atmosphere of 92 per cent. CO and 8 per cent. O₂ (pCO/pO₂ = 11.5) compared with an atmosphere of 92 per cent. N₂ and 8 per cent. O₂, the respiration of pine needles at a temperature of 20°C. is inhibited 70 per cent. This shows that the oxygen-transferring ferment of respiration in plants reacts with CO and must therefore be a compound of a heavy metal.
The inhibition of respiration by CO is reversible in visible light. By alternately illuminating plant cells and placing them in the dark in an atmosphere of O₂ and CO, respiration can be started or stopped. The ferment-CO compound dissociates in the light. This indicates that the oxygen-transferring ferment in plants is a colored substance and that it is a compound of iron.

The absorption of light by the respiration ferment-CO compound is of the same magnitude in plant cells as in liver cells and yeast. The rate of respiration of plum leaves, for example, in an atmosphere of CO/O₂ = 12, in the dark amounts to only 30 per cent. of the respiration in the CO/O₂ atmosphere when the leaves are illuminated by an ordinary 300-watt electric bulb placed 5 cm. below the bottom of the test vessel.

These findings indicate that the oxygen-transferring ferment of respiration in plant cells is identical with the oxygen-transferring phaeohaemin ferment of respiration in animal cells and aerobic bacteria. In white leaves (white ivy, Zebrina pendula, and sections of Coleus), petals, and roots, carbon monoxide did not have an effect of the same magnitude upon respiration.

**Effect of Carbon Monoxide on Respiration in the Dark**

Two to 10 young green tobacco leaves (dry weight 24 to 120 mg.), or 10 to 50 young green pine needles (dry weight 40 to 200 mg.) and similar amounts of plum leaves, Spanish moss, and oleander leaves were put into a conical manometer vessel. Into the insert well of the manometer was poured 0.2 cc. H₂O to supply the requisite humidity, and into the side arm 0.2 cc. of 8 per cent. KOH to absorb the carbon dioxide formed in respiration. The vessels were wrapped in tin foil to exclude light. Gas mixtures of oxygen and carbon monoxide, oxygen and argon, or oxygen and nitrogen were prepared over mercury in a 2-liter gasometer, and the manometer vessels were saturated with these gases. The oxygen consumption was measured at temperatures between 0° and 32° C. in the Warburg apparatus.

Figure 1 shows the respiration of tobacco leaves in the O₂ and N₂ gas mixtures and at various O₂ and CO concentrations; and figure 2 shows the rate of inhibition of respiration in pine needles at various CO/O₂ ratios. Even at a ratio pCO/pO₂ = 4 the respiration is inhibited by 38 per cent. At a ratio of pCO/pO₂ = 20 the inhibition amounts to 77 per cent. If oxygen-argon or oxygen-nitrogen mixtures are substituted for oxygen-carbon monoxide, the respiration rate again becomes the same as that of the control. The inhibition of respiration by CO is therefore totally reversible.

The effect of CO on respiration proved to be independent of temperature in tobacco leaves, pine needles, Spanish moss, and stamens of plums and daffodils. In pistils of daffodils (at pCO/pO₂ = 20) the rate of inhibition increased with increasing temperature (at 14° C. 16 per cent.; at 20° C., 38
Fig. 1. Respiration of tobacco leaves (16 mg.) at various partial pressures of oxygen and carbon monoxide at 32° C.

Fig. 2. Inhibitory effect of CO on respiration of pine needles at different ratios of CO/O₂ at 20° C.
per cent.; at 26° C., 53 per cent.) This dependence of CO inhibition on temperature is probably not due to a greater reactivity between the respiration ferment and CO, but rather to a different degree of saturation of the ferment at different temperatures under the experimental conditions.

**EFFECT OF CARBON MONOXIDE ON RESPIRATION IN LIGHT**

The difficulty in measuring the effect of light on the CO inhibition of respiration in plants is obvious, because as soon as the green plant cells are irradiated by visible light photosynthesis begins. Even if no free carbon dioxide is available, as in the presence of KOH, the green cells form oxygen in the presence of light. This difficulty can be avoided by slightly narcotizing the green cells. As Bernhard (1), and Bonnier and Mangin (2) found, small concentrations of chloroform stop the assimilation without decreasing the rate of respiration. Accordingly, 0.1–0.2 cc. of a 5 per cent. chloroform solution in alcohol was added to the test vessels containing green leaves of the higher plants or pine needles. The time required for complete narcosis of the assimilation depends upon temperature and the variety of plant cells. For instance, 4 to 6 hours, at temperatures of from 10° to 15° C., are necessary to obtain constant gas pressures with pine needles. After this

![Graph](https://www.plantphysiol.org/-/media/pspi/pspi-1936/nature-of-oxygen-transferring-ferment-609.jpg)

**Fig. 3.** Reversibility of carbon monoxide inhibition of respiration of 120 mg. of pine needles in light at 10° C.
period, photosynthesis has ceased and the effect of light upon the CO inhibition of respiration can easily be measured. As source of light, a 300-watt electric bulb was placed 5 cm. below the bottom of the manometer vessel. The vessel was shaken in the thermostat at a temperature of 10° C. Figure 3 gives the result of an experiment which shows the effect of light upon the CO inhibition of the respiration of 120 mg. of pine needles in which assimilation was eliminated by chloroform. While the inhibition of respiration in the dark at a ratio of $p_{CO}/p_{O_2} = 10$ was 67 per cent., the inhibition in the light was only 8.3 per cent. The combination of the respiration ferment with carbon monoxide can be entirely dissociated by visible light.

**Effect of carbon monoxide upon respiration of tobacco leaves with mosaic disease**

The effect of carbon monoxide on tobacco plants infected with mosaic disease has been studied in order to determine whether this virus disease changes the respiratory mechanism of the cells. Small young leaves and sections of leaves showing the spots of the mosaic disease were examined at various CO/$O_2$ ratios. Unless the cells had reached the stage of necrosis, no change was found in the rate of respiration, in the sensitivity of the respiration to CO, and in the sensitivity of the ferment-CO combination toward light.

**Effect of carbon monoxide on plant fermentation**

In the absence of free oxygen various plants can be kept alive for a limited time and photosynthesis may occur (15). The writer has measured the $CO_2$ formation of pine needles in the absence of free oxygen at various temperatures to determine whether this anaerobic metabolism in plants is inhibited by CO, as is the case in some anaerobic bacteria (6, 9) and in the photosynthetic Thiorhodaceae (4, 5). Thirty to 100 mg. of pine needles were put into manometer vessels which were saturated with mixtures of argon and carbon monoxide, argon and carbon dioxide, or carbon monoxide and carbon dioxide. To measure the $CO_2$ consumed in photosynthesis the oxygen was absorbed by white phosphorus in the side arm.

The pine needles can survive the lack of free oxygen at temperatures between 10° and 20° C. for from 4 to 6 hours without losing the capacity to assimilate $CO_2$ when exposed to light. If, within this time, they were brought back to aerobic conditions, the rate of respiration was not diminished. The $CO_2$ formed in the anaerobic-splitting metabolism is about 80 per cent. of the $CO_2$ formed by respiration. Formation of hydrogen in comparable quantities was not found under these conditions. The anaerobic $CO_2$ formation is not influenced by CO.

Since CO inhibits the respiration but not the anaerobic fermentation in plant cells, it is possible even under aerobic conditions to produce or inhibit
fermentation by adding or removing CO. Table I shows the effect of CO on the oxidative and on the splitting metabolism of 85 mg. of pine needles at 20° C. The O₂ absorbed and CO₂ released is measured in the presence and the absence of KOH in O₂ and N₂, O₂ and CO, N₂ and CO. The retention of CO₂ by the plant cells is measured by placing them in the insert well of the manometer vessel and determining the CO₂ which is liberated from a definite amount of bicarbonate contained in the bottom of the main vessel after adding citric acid from the side arm.

<table>
<thead>
<tr>
<th>GASES</th>
<th>O₂ CONSUMED IN RESPIRATION</th>
<th>CO₂ FORMED IN RESPIRATION</th>
<th>CO₂ FORMED IN SPLITTING</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% O₂ - 92% N₂</td>
<td>100</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>8% O₂ - 92% CO</td>
<td>32</td>
<td>32</td>
<td>61</td>
</tr>
<tr>
<td>100% N₂</td>
<td>None</td>
<td>None</td>
<td>77</td>
</tr>
<tr>
<td>100% CO</td>
<td>None</td>
<td>None</td>
<td>77</td>
</tr>
</tbody>
</table>

The quotient, \( \frac{\text{CO}_2 \text{ formed in respiration}}{\text{O}_2 \text{ consumed in respiration}} \), determined by this method is

1. While in an O₂ and N₂ atmosphere a non-oxidative metabolic process does not occur, in an atmosphere of O₂ and CO the CO₂ formed by splitting amounts to 80 per cent. of the CO₂ formed under anaerobic conditions.

Summary

1. The purpose of the work reported in this paper was to determine the chemical nature of the oxygen-transferring ferment in plants, and its possible identity with one of the two oxidation ferments already known, that is, with the iron-free yellow ferment which is insensitive to carbon monoxide, or phaeohaemin which is sensitive to carbon monoxide.

2. The effect of carbon monoxide on the respiration of stamens of plums, stamens and pistils of daffodils, Spanish moss, green pine needles, and green leaves of tobacco, plum, and oleander plants has been examined. It was found that the oxygen-transferring ferment of respiration in plants is a compound of a heavy metal, for in all these plant cells carbon monoxide produces a strong reversible inhibition of respiration, and instead of the respiratory metabolism a non-oxidative splitting metabolism appears. The oxygen-transferring ferment in plants is a compound of iron; for, the ferment-CO compound dissociates in light; the carbon monoxide inhibition of respiration
ceases if the cells are illuminated, and respiration again takes the place of the non-oxidative splitting metabolism.

3. The sensitivity of the respiration ferment toward carbon monoxide, and the absorption of light by the respiration ferment-CO compound are of the same magnitude in the cells of the higher plants as in animal cells and aerobic bacteria. All these findings indicate that the oxygen-transferring ferment of respiration in plant cells is identical with the oxygen-transferring ferment phaeohaemin.

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LITERATURE CITED


