INHIBITION OF RESPIRATION AND PHOTOSYNTHESIS IN CHLORELLA PYRENOIDOSA BY ORGANIC COMPOUNDS THAT INHIBIT COPPER CATALYSIS*

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The suggestion that compounds known to inhibit copper catalysis might exert an inhibiting effect upon photosynthesis and respiration in green plants arose primarily from the following considerations: (a) The aerobic oxidation of ascorbic acid is very sensitive to catalysis by copper-protein complexes in a great variety of plant press-juices (1). (b) Both copper (2) and ascorbic acid (3) are found in relatively high concentrations in green leaves and other plant tissues that have a capacity for photosynthesis. (c) Although the evidence is far from complete, it is not unreasonable to believe that copper and ascorbic acid may be concerned, directly or indirectly, with both respiration and photosynthesis in green plants.

Previous work in other laboratories has shown that photosynthesis is more sensitive than respiration to inhibition by cyanide (4–6) and the urethanes (6, 7).

The present investigation has shown that typical copper “poisons” inhibit both photosynthesis and respiration in Chlorella cells, the respiration being less sensitive than photosynthesis.

EXPERIMENTAL

The unicellular fresh water alga Chlorella pyrenoidosa was grown in pure culture and used for the experiments primarily because of the extensive investigations that have been made on photosyn-

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Respiration and Photosynthesis

thesis and respiration in these plants, and further, because of the convenience of their use in manometric vessels. The strain selected has been in use for a number of years by one of us (8, 9) as well as by others (5-7, 10-13), for photosynthesis experiments.

The method of culture was essentially the same as that used by the authors cited. Flasks containing sterile salt solutions were inoculated and placed in a water bath close to an ordinary incandescent lamp (60 watt). A slow continuous stream of 5 per cent carbon dioxide in air was passed through the flasks. The temperature of the water bath was maintained at about 25°. The cultures were used after they had grown for 2 or 3 weeks, at which time they contained about 4 c.mm. of cells (centrifuged) per ml. of fluid.

The manometric method of Warburg (6) was used to determine the rates of respiration and of photosynthesis during short periods. Vessels of the rectangular type were employed, with a fluid volume of 8 ml. For the experiments the Chlorella cells were centrifuged out of their culture fluid and suspended in 0.05 M KH₂PO₄, pH 4.6, which was not altered when the suspension was saturated with 5 per cent CO₂ in air for the photosynthesis experiments. This phosphate-CO₂ medium has been found very favorable for measurement and calculation of maximum photosynthesis rates (9).

For attaining the high light intensities required to promote a maximum rate of photosynthesis, incandescent lamps (60 watt) were immersed in the thermostat very close to the vessels, one lamp for each vessel. This was found to give adequate intensity, but made the control of temperature difficult at 25°. Because of this, most of the experiments, including all of those specifically cited here, were performed at 30°. Control experiments showed a constant rate of photosynthesis over a period of at least 2 hours. In the respiration experiments the vessels were covered with tin-foil.

The calculation of rates is based on the assumption that the respiratory and photosynthetic quotients (−CO₂/O₂) are unity. This is known to be true in the absence of the inhibitors (6, 14, 15). The inhibitors may have some effect on these quotients and in turn on the recorded quantitative rates, but the error would probably not alter the interpretation of our results. Assuming unity of these quotients, the amount of O₂ produced or consumed (c.mm.)
is equal to $K_1 \times h$, where $h$ is the pressure change in mm. and $K_1$ is the vessel constant for a quotient of unity, equal to $k_{CO_2} \times$

![Fig. 1](http://www.jbc.org/)

**Fig. 1**. Inhibition of photosynthesis in *Chlorella* by thiourea. Temperature 30°, pH 4.6, 0.05 M KH$_2$PO$_4$, 20 c.mm. of cells, 8 ml. total fluid volume per vessel. Curve A, control, no inhibitor; Curve B, 0.035 mM of inhibitor; Curve C, 0.07 mM of inhibitor; Curve D, 0.14 mM of inhibitor.

**Fig. 2**. Inhibition of photosynthesis in *Chlorella* by 8-hydroxyquinoline. Temperature 30°, pH 4.6, 0.05 M KH$_2$PO$_4$, 20 c.mm. of cells, 8 ml. total fluid volume. Curve A, control, no inhibitor; Curve B, 0.01 mM of inhibitor; Curve C, 0.001 mM of inhibitor.

$k_{O_2}/k_{CO_2} = k_{O_2}/k_{CO_2}$ and $k_{O_2}$ being the vessel constants for a single gas, calculated in the usual manner (14, 15).

**Effect of Inhibitors**—Typical photosynthesis experiments are shown in Figs. 1 and 2. Each curve represents a separate vessel,
the volume of cells in each vessel being 20 c.mm. (centrifuged). The amounts of thiourea and of 8-hydroxyquinoline shown are the total amounts in 8 ml. of fluid. The inhibitors were added to the cell suspensions about 50 minutes before the pressure readings were begun.

For the measurement of respiration a much larger volume of cells was required, as the respiratory rate of the Chlorella cells was about 2.5 per cent of the photosynthesis rate.

The amounts of inhibitors required to inhibit respiration were larger than required for inhibiting photosynthesis, partly because of the larger number of cells present, and partly because of a lower sensitivity of respiration to the inhibitors. Small amounts of the inhibitors were either without effect or produced an acceleration of respiration. Fig. 3 illustrates the effect of thiourea on respiration. The thiourea was added to 100 c.mm. of cells 35 minutes before the readings were begun. The amounts of inhibitors shown are the total amounts in 8 ml. of fluid.

Because of the low solubility of 8-hydroxyquinoline, it was not possible to obtain a solution concentrated enough to inhibit respiration. A saturated solution gave a rate identical with that of the control.
The other inhibitors tried, potassium ethyl xanthate, sodium diethyldithiocarbamate, allylthiourea, and salicylaldoxime, all gave marked inhibitions of photosynthesis when similar amounts of the inhibitors were present. The effects of xanthate and carbamate on respiration were obscured by the evolution of gas in the presence of large amounts of Chlorella cells. This was sufficient to overbalance the negative pressure change due to respiration, and was apparently due to a slow decomposition of these rather unstable organic substances.

**Reversibility of Inhibitions**—Studies were made concerning the
reversibility of thiourea and salicylaldoxime inhibition on photosynthesis and respiration. Aliquot portions of the necessary volume of culture suspended in 0.05 M KH₂PO₄ (pH 4.6) were used. One was poisoned, then dialyzed against water distilled in Pyrex vessels. Another dialyzed similarly but without the addition of an inhibitor, served as a control. A third portion was used as an undialyzed control, for comparison with the fourth portion to which the inhibitor was added. The results with thiourea as an inhibitor of photosynthesis are illustrated in Fig. 4. The inhibitor was left in contact with the cells for 30 to 40 minutes before dialysis was begun. A period of 4 hours of dialysis in

**Table I**

**Reversibility of Salicylaldoxime Inhibition of Chlorella Respiration and Photosynthesis**

Temperature 30°, pH 4.5, 0.05 M KH₂PO₄. The results are given in c.mm. of O₂ per hour.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Respiration</th>
<th>Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibited</td>
<td>After dialysis</td>
</tr>
<tr>
<td>None (initial)</td>
<td>45</td>
<td>63</td>
</tr>
<tr>
<td>Salicylaldoxime*</td>
<td>8</td>
<td>61</td>
</tr>
</tbody>
</table>

* The amount of salicylaldoxime used in the respiration experiments was 0.043 mM with 100 c.mm. of cells; in photosynthesis, 0.014 mM with 20 c.mm. of cells. These quantities represent the total amounts in 8 ml., the total volume in the vessels.

cellulose (Visking) tubes, with frequent changes of water, was sufficient to remove the inhibitors to non-effective concentrations. The cell suspensions were centrifuged and brought to their original volumes with 0.05 M KH₂PO₄ before being placed in the vessels. The reversibility of salicylaldoxime inhibition is shown in Table I. As can be seen from Fig. 4 and Table I, dialysis (alone) of the *Chlorella* suspension caused slight increases in rates of respiration and photosynthesis.

A number of titrations (3, 16) of the ascorbic acid content of the centrifuged *Chlorella* cells gave an average value of 0.17 mg. per ml., without marked variation in the samples studied.
SUMMARY

1. A series of organic compounds characterized by their activity as copper "poisons," thiourea, 8-hydroxyquinoline, allylthiourea, sodium diethyldithiocarbamate, potassium ethyl xanthate, and salicylaldoxime all exhibited marked inhibition of photosynthesis in Chlorella pyrenoidosa.

2. The respiration of Chlorella pyrenoidosa was also inhibited by thiourea and salicylaldoxime, although higher concentrations were required, as found previously for cyanide inhibition. The solubility of 8-hydroxyquinoline was too low to permit a marked effect upon respiration. The effect of sodium diethyldithiocarbamate and potassium ethyl xanthate on respiration was obscured by the effect of their decomposition products.

3. The inhibition of both respiration and photosynthesis in Chlorella pyrenoidosa by thiourea and salicylaldoxime was reversible. The initial rates were fully restored after 4 hours dialysis and centrifuging.

4. The ascorbic acid content of centrifuged cells of Chlorella pyrenoidosa was 0.17 mg. per ml.

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