LIGHT-DEPENDENT OXYGEN METABOLISM OF CHLOROPLAST
PREPARATIONS. II. STIMULATION BY MANGANOUS IONS 1, 2
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The importance of manganese for photosynthesis was first suggested by McHargue (17) and Bishop
(2). Pierson (21) found that photosynthesis in manganese-starved Chlorella cells was inhibited despite
the absence of visible chlorosis; this inhibition could be relieved by adding manganese to the medium.
Emerson and Lewis (6) observed that manganese deficiency affected the maximum quantum yield of
photosynthesis. Gerretsen (7) found that manganese deficiency caused a reduction in the rate of photo-
synthesis in oats. He surmised that manganese is specifically involved in the oxygen-liberating stage of
photosynthesis and went on to study the effect of manganese salts on the potentials of electrodes im-
mersed in chloroplast suspensions (8). Working with chloroplasts in the absence of Hill reagents, Ger-
retsen found that the electrode potential was changed very markedly on addition of manganese and he sug-
gested that peroxide is formed in the presence of manganese but not in its absence.

More recently Kessler (15, 16), from his studies of photosynthesis and photooxidation in manganese-
deficient algae, provided convincing proof that the role of manganese in photosynthesis is mainly, if not
exclusively, concerned with oxygen evolution.

Aside from the implication of manganese, we know very little about the process by which oxygen is
evolved from the oxidized product of the splitting of water in photosynthesis. There is a comparable lack
of experimental evidence concerning the role of manganese in photochemical oxygen production by isolated
chloroplasts.

Mehler (18) was the first to show that oxygen could function as a Hill reagent, i.e., as an oxidant
which is effective in promoting photochemical oxygen production by chloroplast preparations. In the
Mehler reaction there is a light-dependent consumption of oxygen which is a consequence of the simul-
taneous reduction of oxygen by a photochemically generated reductant and evolution of oxygen from the
oxidized product of photolysis. Added ethanol and excess catalase act as a "sink" for the peroxide pro-
duced by the reduction of molecular oxygen (13). The stoichiometry of the over-all reaction is such
that two molecules of oxygen are consumed for every one produced (Mehler and Brown, 20).

More recently, Brown and Good (3) have shown that chloroplasts illuminated without addenda can
produce and consume oxygen simultaneously. Both production and consumption are light dependent
processes. The stoichiometry being 1:1, the chloro-
plasts thus effect an exchange of the oxygen of water for molecular oxygen dissolved in the suspending
medium. In this exchange reaction (which differs from the Mehler reaction only in the method of re-
moval of peroxide formed in the reduction of molecular oxygen), each mole of oxygen reduced by chloro-
plast preparations in the light is exactly balanced by the production of 1/2 mole from the oxidized product
of photolysis and 1/2 mole from the decomposition of
peroxide by the endogenous catalase of the chloro-
plasts. Because there is no net change of oxygen
tension in the exchange reaction, it can be followed
only by tracer oxygen techniques.

Mehler (19) tested the effect of added manganous
ions on the Hill reaction and found no effect on the
rate of oxygen evolution when quinone was used as
the oxidant (whether or not catalase and ethanol were
present). In the case of the Mehler reaction, how-
ever, he found that Mn** increased the rate of net
oxygen consumption. The manganese-stimulated
rates were approximately the same as those which
Mehler (19) had observed following quinone reduc-
tion. This stimulatory effect of manganese was cata-
litic; marked stimulation was given by 10^{-4}M
Mn** and the maximum effects were produced by a
10^{-3} M concentration.

Andreae (1) has reported a flavin-sensitized light-reduction of manganese ions and has suggested
that the cyclic oxidation-reduction of manganese might explain the effects of manganese ions on oxy-
gen uptake by illuminated chloroplast suspensions. Also, Kenten and Mann (14) have demonstrated
a light-dependent oxidation of manganese in chloroplast preparations and have discussed its possible role in
photosynthesis.

Mehler's observation of the manganese stimulation
has been confirmed by Good and Hill (9) and ex-
tended to preparations from chard and Chenopodium.

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1 Received July 16, 1959.
2 The contents of this paper are part of a thesis sub-
mited to the graduate faculty of the University of Minne-
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Fig. 1 (top left). Effect of Mn\(^{++}\) concentration on rates of net oxygen uptake in the Mehler reaction. Vessel contents: 3 cc chloroplast suspension (containing 0.5 mg chlorophyll), 6 mg catalase, 0.1 cc 50 % ethanol, 0 to 4 μM MnCl\(_2\).

Fig. 2 (top right). Effect of adding Mn\(^{++}\) on time courses of normal and tracer oxygen in the Mehler reaction (Exp. 1). Vessel contents: 3 cc chloroplast suspension (containing 0.75 mg chlorophyll), 6 mg catalase, 0.1 cc 50 % ethanol, 6 μM MnCl\(_2\) in sidearm. To convert partial pressure units of the ordinate to microliters oxygen multiply by 1.12.
They have suggested that increased oxygen uptake must have been due to hydrogen peroxide formation because it did not occur when the hydrogen peroxide-trapping catalase and ethanol were omitted.

This paper describes experiments in which tracer oxygen techniques were used to measure the effects of manganous ions on the rates of consumption and production of oxygen in the Mehler reaction and the related exchange reaction. The data indicated that manganese stimulation resulted from a reaction with the oxidized product of photolysis and there was indirect evidence of peroxide formation. The stimulation of light-dependent chloroplast reactions by manganous ions is compared with the stimulation by quinone and ascorbic acid previously described (Habermann, 10; Habermann and Brown, 11) and a scheme for the action of manganese is proposed.

METHODS

Chloroplasts were prepared from mature leaves of Phytoleca americana L. according to procedures previously described (Habermann, 10). All reactions were run in conventional rectangular Warburg vessels. Light from a bank of fluorescent bulbs mounted vertically behind the water bath was reflected onto the bottoms of the experimental vessels by a mirror mounted at a 45° angle. Incident light intensity was approximately 400 ft-c. Manometric experiments were run at 15.6° C. isotope experiments at either 14.5° or 23° C. Adaptation of the mass spectrometer (CEC model 21-201) has been described by Brown, Nier, and Van Norman (4), and further modifications of the apparatus have been described by Johnston and Brown (12).

The gas phase for all manometric experiments was air. In experiments using tracer oxygen, the vessel containing 3 cc buffered chloroplast suspension plus addenda was attached to the leak assembly of the mass spectrometer and flushed with helium for 5 minutes. Oxygen enriched with mass 34 (O^{16}O^{18}) was then added so that the resulting gas phase was composed of 2 to 3% oxygen in helium. The rates of oxygen consumption and evolution were calculated from time course records of the concentrations of mass 32 (ordinary oxygen) and mass 34 (O^{16}O^{18}) according to methods discussed by Brown and Weis (5).

RESULTS

Effect of Mn++ Concentration: Figure 1 shows the rate dependence of the manganese-stimulated Mehler reaction on the amount of Mn++ added to the reaction mixture. Considerable stimulation of net oxygen uptake was observed at even the lowest concentration of added manganous ions and maximally stimulated rates were observed at concentrations above 10^{-4} M in the reaction mixture.

Tracer Oxygen Experiments: I. Effect of Mn++ on the rates of oxygen consumption and production in the Mehler reaction: A number of experiments were run in which MnCl_2 in solution was tipped from the sidearm of the experimental vessel after a period of unstimulated Mehler reaction in the light. The time courses of uptake of tracer and normal oxygen in a typical experiment are shown in figure 2. Following the addition of manganous ions to the reaction mixture, the rates of net consumption of both species were accelerated. The calculated rates of oxygen consumption and production in this experiment are given in table I (Exp. 1). Also in table I (Exp. 2) are data of a second representative experiment run with a different chloroplast preparation and at a higher temperature. In both experiments, the rates of uptake and production of oxygen in the Mehler reaction were accelerated following addition of Mn++ ions to the reaction mixture. In this case, uptake was stimulated much more than production (the rate of oxygen uptake increased an average of 83% after adding Mn++, while the rate of oxygen production increased only 40%).

II. Effect of Mn++ on the rates of uptake and production of oxygen in the exchange reaction: Experiment 3 (table I) was run with an aliquot of the chloroplast suspension used in experiment 1. Three cc of chloroplast suspension was pipetted into the main compartment of the vessel and MnCl_2 in solution was tipped from the sidearm after a period of unstimulated oxygen exchange in the light. The time courses of tracer and normal oxygen pressure changes in this experiment are shown in figure 3 and the calculated rates of oxygen consumption and production are summarized in table I (Exp. 3). In this case also, the rates of both uptake and production of oxygen were accelerated by the addition of manganous ions. In the exchange reaction, however, in contrast to the changes observed in the Mehler reaction, production of oxygen was stimulated more than uptake (the rate of uptake increased only 34%, while the rate of production increased 61%).

Figure 3 (bottom left) Effect of adding Mn++ on time courses of normal and tracer oxygen in the exchange reaction (Exp. 3). Vessel contents: 3 cc chloroplast suspension (containing 0.75 mg chlorophyll), 6 μM MnCl_2 in sidearm. To convert partial pressure units of the ordinate to microliters oxygen multiply by 1.53.

Figure 4 (bottom right) Effect of Mn++ concentration on rates of oxygen production in the quinone reaction and on rates of net oxygen consumption of the subsequent quinone-stimulated Mehler reaction. Vessel contents: 3 cc chloroplast suspension (containing 0.5 mg chlorophyll), 6 mg catalase, 0.1 cc 50% ethanol, 6 μM quinone, 0 to 8 μM MnCl_2.
Table I
EFFECTS OF ADDED MANGANOUS IONS ON RATES OF OXYGEN UPTAKE AND PRODUCTION IN MEHLER AND EXCHANGE REACTIONS

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th></th>
<th></th>
<th>Exp. 2</th>
<th></th>
<th></th>
<th>Exp. 3</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxygen uptake (μL/min)</td>
<td>Oxygen production (μL/min)</td>
<td>Net rate (μL/min)</td>
<td>Oxygen uptake (μL/min)</td>
<td>Oxygen production (μL/min)</td>
<td>Net rate (μL/min)</td>
<td>Oxygen uptake (μL/min)</td>
<td>Oxygen production (μL/min)</td>
</tr>
<tr>
<td>Initial dark period</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>−0.27</td>
<td>+0.01</td>
<td>−0.26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unstimulated light reaction</td>
<td>−1.18</td>
<td>+0.44</td>
<td>−0.74</td>
<td>−1.64</td>
<td>+0.69</td>
<td>−1.04</td>
<td>−1.48</td>
<td>+1.34</td>
</tr>
<tr>
<td>Mn⁺⁺ stimulated light reaction</td>
<td>−2.27</td>
<td>+0.69</td>
<td>−1.58</td>
<td>−2.86</td>
<td>+0.74</td>
<td>−2.12</td>
<td>−1.98</td>
<td>+2.16</td>
</tr>
<tr>
<td>Final dark period</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Increase in rate after addition of Mn⁺⁺</td>
<td>92 %</td>
<td>57 %</td>
<td>114 %</td>
<td>74 %</td>
<td>23 %</td>
<td>104 %</td>
<td>34 %</td>
<td>61 %</td>
</tr>
</tbody>
</table>

Vessel contents and experimental conditions: Exp. 1. 3 cc chloroplast suspension (containing 0.75 mg chlorophyll), 6 mg catalase, 0.1 cc 50 % ethanol, 6 μM MnCl₂ in sidearm; temp. 14.5⁰ C. Exp. 2. 3 cc chloroplast suspension (containing 0.42 mg chlorophyll), other addenda the same as in Exp. 1; temp. 23⁰ C. Exp. 3. 3 cc chloroplast suspension (containing 0.75 mg chlorophyll), 6 μM MnCl₂ in sidearm; temp. 14.5⁰ C.

Experimental data summarized in figure 4 show the effects of increasing amounts of manganous ions in reaction mixtures containing identical amounts of chloroplast suspension, quinone, catalase, and ethanol. Low concentrations of added Mn⁺⁺ had no effect on either the rate of oxygen evolution in the quinone reaction or on the rate of net oxygen consumption in the Mehler reaction which followed quinone reduction. When amounts of Mn⁺⁺ greater than 4 micromoles were added, however, the rates of oxygen production in the quinone reaction were reduced by approximately 20 %.

Discussion
Stimulation of the Mehler reaction by manganous ions is similar in its effect on net oxygen uptake to the effects of quinone and ascorbic acid. Although net uptake of molecular oxygen is stimulated by all three substances, their effects on the actual rates of uptake and production of oxygen are different. These differences, then, must serve as the basis for any hypothesis concerning the nature of their action. In contrast to quinone, which stimulates the rates of uptake and production equally so that the stoichiometry of the over-all reaction remains unchanged (10), both ascorbic acid (11) and manganous ions produce an unequal stimulation of oxygen consumption and production with greater increments in the rates of oxygen uptake. Unlike the ascorbic acid effect, which lasts only so long as unoxidized ascorbic acid remains in the reaction mixture and in which the net amount of oxygen taken up in excess of the unstimulated rate is equivalent to the ascorbic acid initially added (11), there is no tendency for manganous-stimulated rates to revert to those of the controls. In this respect, manganous stimulation resembles that of quinone. Stimulated rates have been followed for several hours using the stable chloroplasts prepared from Phyloeca.

The catalytic nature of the manganous stimulation and the unequal stimulation of the rates of consumption and production in no way argue against a cyclic oxidation and reduction of manganese such as that proposed by Andreae (1) and also by Kenten and Mann (14). A chlorophyll-sensitized photooxidation of manganous ions superimposed on the Mehler reaction might be plausible via a scheme analogous to that proposed by Andreae (1) for his non-biological system. This is improbable, however, because of the observed enhanced rates of oxygen production. A more likely reaction mechanism would involve an alternate pathway mediated by Mn⁺⁺ in the reaction sequence of the oxidized product of photolysis. Such a pathway could conceivably lead to oxygen production via peroxide formation. Under the conditions of the Mehler reaction, peroxide thus formed would be removed in the coupled oxidation of ethanol to acetaldehyde. A more efficient removal of the oxidized product would decrease the probability of a back reaction, or recombination of the products of photolysis. The enhanced reduction of molecular oxygen by the reduced product of photolysis could thus be a consequence of the more efficient removal of one of the products of photolysis. This reaction mechanism is consistent with all the experimental data.

Let us then examine the experimental results in terms of the proposed mechanism: Adding manganous ions to a Mehler reaction mixture resulted in increased rates of net oxygen uptake which could be
observed manometrically. Tracer studies showed
that this enhanced rate of net uptake was the result of
a stimulation of the rate of oxygen uptake which was
considerably greater than the stimulation of its rate
of production. In the presence of manganous ions,
removal of the oxidized product of photolysis (other
than by back reaction with the reduced product) could
follow two possible pathways: i.e., the usual pathway
to oxygen of the unstimulated Mehler reaction or
peroxide formation mediated by manganous ions. An
effective peroxide trapping mechanism (the catalase
and ethanol in the Mehler reaction mixture) removes
the peroxide thus formed. In accordance with the
proposed mechanism then, uptake was enhanced and
the ratio of uptake to production was greater than in
the unstimulated reaction.

Adding manganous ions to chloroplast prepara-
tions with no other addenda (which on illumination
carry on an oxygen exchange reaction) resulted once
again in enhanced rates of both uptake and production
of oxygen. In this case, the peroxide trap had not
been added to the reaction mixture but the endogenous
catalase of the chloroplasts was able to decompose
peroxide catalatically by dismuting it to H₂O and O2
The ratio of uptake to production should theoretically
have remained constant because the alternate path-
way for the oxidized product of photolysis results in
oxygen production, as does the normal pathway.
The calculated rates of oxygen uptake and production
showed that their ratio changed from slightly more
to slightly less than the theoretical value of one.

According to the proposed reaction mechanism,
the presence of manganous ions in a Hill reaction mix-
ture would not enhance the rate of oxygen produc-
tion. Quinone seems to be efficient in its reaction
with the reduced product of photolysis. Evidence
for this is the observation that the rates of oxygen
production with quinone as the oxidant are as a rule
twice to several times those observed when oxygen
is used as the Hill reagent. If a peroxide trap were
present in the reaction mixture, however, we would
anticipate that manganous-mediated peroxide produc-
tion from the oxidized product of photolysis could
decrease the rate of oxygen production. When catalase
and ethanol were present during the quinone reaction
and sufficiently high concentrations of Mn²⁺ were
added, a decrease in the rate of oxygen production
was observed.

These experiments support the possibility that
manganese-mediated peroxide formation is involved
in the evolution of oxygen in photosynthesis. They
do not, however, tell us whether oxygen evolution
in vivo proceeds via peroxide formation similar to
that which is indicated in vitro, or by the apparently
normal pathway to oxygen which exists in unstimu-
lated chloroplast preparations.

**Summary**

Tracer oxygen techniques were used to measure
simultaneously the rates of consumption and produc-
tion of oxygen in unstimulated and manganese-stim-
ulated light-dependent chloroplast reactions. Both
rates were stimulated on addition of manganous ions
to the Mehler system and a similar stimulation of the
related exchange reaction was observed.

These experiments indicate that the action of man-
ganese is primarily involved with the oxidized prod-
uct of photolysis. A reaction mechanism has been
postulated whereby manganese mediates peroxide for-
mation from the (OH)-product of the splitting of
water. This provides an alternate pathway which re-
sults in the more efficient removal of this product and
accounts for the stimulation of net oxygen uptake by
manganese ions.

**Acknowledgements**

The author wishes to thank Prof. Allan H. Brown
for his help and encouragement during the course of
experimentation. These studies were aided through
funds supplied by the Graduate School of the Univer-
sity of Minnesota and also by a contract between the
Office of Naval Research, Department of the Navy
and the University of Minnesota (NR 104 030).
Tracer oxygen was obtained from Prof. A. O. C. Nier
and was prepared under a grant from the American
Cancer Society, through the Committee on Growth
of the National Research Council. During the sum-
er of 1955 this work was encouraged by the financial
assistance of the Lawrence S. Moyer fellowship. It is
with deep gratitude that this support is acknowl-
dged.

**Literature Cited**

1. Andreake, W. A. 1955. The photoinduced oxida-
55: 584–586.

2. Bishop, W. B. S. 1928. The distribution of man-
ganese in plants and its importance in plant metabo-
125–141.

reduction of oxygen in chloroplast preparations and
in green plant cells. I. The study of oxygen ex-
changes in vitro and in vivo. Arch. Biochem.

Norman. 1952. Measurement of metabolic gas
exchange with a recording mass spectrometer.

5. Brown, A. H. and D. Weis. 1959. Relation be-
tween respiration and photosynthesis in the green
alga Ankistrodesmus braunii. Plant Physiol. 34:
224–234.

6. Emerson, R. and C. M. Lewis. 1940. The quan-

photosynthesis. I. Carbon dioxide assimilation and
the typical symptoms of manganese deficiency in

photosynthesis. II. Reduction-oxidation potentials
of illuminated crude chloroplast preparations.
Plant and Soil. 2: 159–193.


