THE PHOTOSYNTHETIC FUNCTION OF MANGANESE AND CHLORIDE

HOWARD A. TANNER, THOMAS E. BROWN, CLYDE EYSTER AND R. W. TREHARNE

Charles F. Kettering Foundation, Yellow Springs, Ohio

A careful comparison of the effects of micronutrient concentrations on heterotrophic and autotrophic cultures of various algae and aquatic plants revealed definite manganese and chloride requirements for autotrophic growth, suggesting that these elements have a specific photosynthetic function (Eyster et al., 1958). Since it is possible to produce plants which are deficient in manganese but otherwise normal, information on the photosynthetic role of manganese can be obtained by comparative studies of normal and manganese deficient plants.

One interesting result of such studies is that manganese deficient plants, which show no Hill reaction oxygen production, will still produce oxygen photosynthetically at about a third the rate of normal plants (Brown et al., 1958). This raises the very interesting possibility that there are two paths for oxygen production, one of which requires manganese and is solely responsible for Hill reaction oxygen production. Additional work has confirmed this and it appears that the major photosynthetic processes are the following:

$$ADP + H_3PO_4 \rightarrow ATP + [H] + [OH] \quad (1)$$

$$2ADP + 2H_3PO_4 + TPN^+ \rightarrow 2ATP + TPNH + H^+ + H_2O + \tfrac{1}{2}\, O_2 \quad (2)$$

$$TPNH + H^+ + HCO_3^- + MnCl^+ \rightarrow [CHO] + TPN^+ + MnCl(OH)_2 \quad (3)$$

$$H^+ + MnCl(OH)_2 \rightarrow MnCl^+ + 1\tfrac{1}{2}\, H_2O + \tfrac{1}{4}\, O_2 \quad (4)$$

$$2[CHO] \rightarrow [(CHO)_2] \rightarrow CH_2OHCOOH \quad (5)$$

With suitable cofactors reaction 1 is "cyclic" photophosphorylation and 2 is "non-cyclic" photophosphorylation (Arnon, 1959). Reaction 2 provides TPNH and ATP for the operation of the Calvin photosynthetic cycle (Arnon, 1958; Bassham and Calvin, 1957). Reaction 3 depends on the spontaneous alkaline oxidizability of $MnCl^+$ to $MnCl^{++}$ due to liberation of OH^- when HCO_3^- is reduced to a neutral intermediate. At some more acid location, perhaps an aerobic oxidation site, the spontaneous reverse reaction 4 occurs. The reduced intermediate [CHO] forms glycolic acid, perhaps via glyoxal, in reaction 5. Reaction 3, as written, has a calculated free energy change of about zero. It is probably coupled with a phosphate energy transfer to give a reasonable rate. Reactions 3 and 4 together would constitute the Hill reaction. The rate of Hill oxygen evolution from *Chlorella* cells in a carbonate free medium is doubled in 1.4% CO_2 in argon compared to the rate in pure argon (Warburg, 1958), a result consistent with reaction 3.

Reactions 3, 4 and 5 constitute a system for oxygen production and carbon dioxide fixation which is locally irreversible because the oxidation and reduction of manganese occur at two different sites where the pH is suitable. The irreversible removal of TPNH assists the photochemical system in the forward direction.

This system constitutes an alternate path for photosynthetic carbon to the Calvin cycle. It is interesting to note that the existence of two photosynthetic fixation paths has been suspected because of pH effects (Ouellet and Benson, 1952), a colored light effect (Cayle and Emerson, 1957), and the inhibitory effect of ethanol (LeFrancois and Ouellet, 1959).

The relative importance of the two paths would vary considerably with physiological conditions. Since the Calvin cycle also produces glycolic acid (Schou et al., 1950; Griffith et al., 1959), both systems produce the same metabolic products but the proportions will be different for short time labeling experiments.

The photosynthetic oxygen production rates suggest that the two paths are comparable. Since photosynthetic growth is logarithmic, a doubling of the growth rate produces a very large difference in total growth after several cycles. Manganese deficient algae cultures show virtually no growth in a week compared to the amount of growth in normal cultures.

The proposed mechanism is supported by several kinds of experimental evidence.

The spontaneous oxidation in vitro of 10^{-5} M $MnCl^{+}$ to $MnCl(OH)_2$ at pH 10, and its reversal at pH 7, are shown by the Electron Spin Resonance (ESR) signals (fig. 1). In the absence of chloride the reversal requires a very much lower pH. This quick reversal in vitro is not perfect but does show the feasibility of a pH controlled cycle in vivo. A light induced alteration of manganese in *Chlorella* is indicated by the rate at which it is washed out of living cells in manganese free Warburg and Burk media. There is a 5-fold increase in the light over the dark.

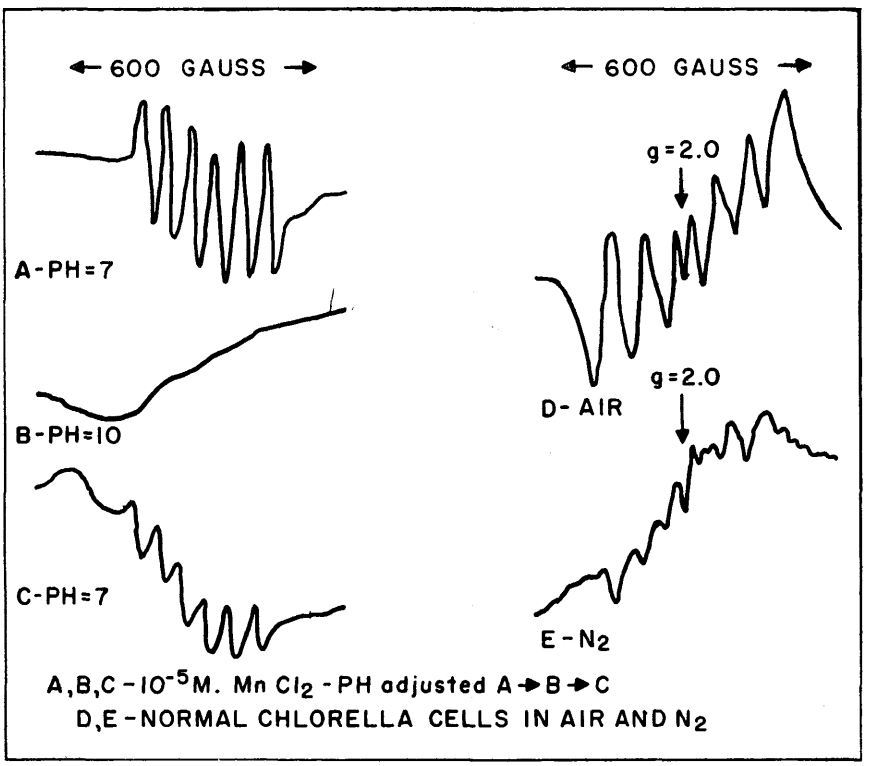

FIGURE 1. Electron spin resonance signals (ESR).

In nitrogen, the manganese ESR signal from normal *Chlorella* cells is very weak (fig. 1). Oxygen thus seems to facilitate reaction 4, probably by producing sites of low pH value through aerobic oxidation processes.

The appearance of a light induced photosynthetic free radical ESR signal is manganese dependent and the Mn^{++} signal decreases reversibly in the light (Treharne et al., 1960). This decrease occurs slowly and recovery is slow, compared to the oxygen liberation rate or the rate of the free radical formation and disappearance.

The rate of $C^{14}O_2$ photosynthetic uptake was found to be twice as fast for normal *Chlorella pyrencidosa* as for manganese deficient cells. The distribution of products labeled photosynthetically by $C^{14}O_2$ was investigated by conventional

TABLE 1

Mn dependent $C^{14}O_2$ photosynthetic products

	Air+2% $C^{14}O_2$		N_2+1% $C^{14}O_2$	
	+Mn	−Mn	+Mn	−Mn
Glycolic acid	1050	43	202	123
Malic acid	1320	110	1480	824

Counts per minute per mg of chlorophyll. 3500 ft-c, white fluorescent light.

TABLE 2

Effect of light on uptake of C^{14} compounds by +Mn and −Mn Chlorella pyrenoidosa

	−Mn and light	−Mn and dark	+Mn and light	+Mn and dark
1—C^{14} Glycolate	19,500	18,400	78,100	23,100
C^{14} Formate	555,700	24,850	114,500	30,600
C^{14} Oxalate	271,180	3,350	75,220	3,270
3—C^{14} Glycerate	41,546	24,593	14,385	26,124
C^{14} Glyoxylate	50,500	59,400	50,200	54,400

Counts per minute/(mg of chlorophyll ×2hr).

TABLE 3

Hill reaction and photophosphorylation by sugar beet chloroplasts

	−Mn	+Mn
Chlorophyll (mg/ml of prep.)	.519	1.314
Hill Reaction [μl O_2/(hr×mg chlorophyll)]	29.	813.
Phosphate uptake [μmole /(hr×mg chlorophyll)]	24.8	17.0

TABLE 4

Reduction of TPN by Grana preparation and chloroplast extract

Chloroplast Extract Source	Grana from −Mn sugar beets	Grana from +Mn sugar beets
Market spinach	24.4	14.2
+Mn sugar beets	25.9	29.4
−Mn sugar beets	24.6	24.7

μmole TPN reduced/(mg chlorophyll×hr).

procedures (Benson et al., 1950). The results were similar to those in the literature, and were rather indifferent to the presence of manganese, with the exceptions of glycolic acid and malic acid. Both of these compounds are much more heavily labeled in normal cells than in manganese deficient cells (table 1).

The effects of light and manganese on the uptake rate of several C^{14} labeled metabolites were determined (table 2). The uptake of glycolate is increased by light and by manganese. Glycolate is first oxidized to glyoxylate (Tolbert and Cohan, 1953); hence, the stimulation is associated with an oxidation reaction. Formate, oxalate, glyoxalate, and glycerate uptakes are increased by light and by absence of manganese. These are reductive assimilations and manganese would compete with them for light induced TPNH (reaction 3). The labeled products formed from these metabolites were determined by the usual chromatographic procedures and were found to be consistent with the sequence $CO_2 \rightarrow$glycolate$\rightarrow$ glyoxylate$\rightarrow$malate, serine, etc.

The glycolate to glyoxylate oxidation could consume all of the oxygen produced by reaction 2. This oxygen source may be physically favored and would help to keep the photochemical process moving in the forward direction. The accumulation of glycolate shows that there is no close chemical coupling between oxygen from reaction 2 and glycolate oxidation.

Glycolic acid is one of the earliest labeled products of $C^{14}O_2$ photosynthesis (Bassham and Calvin, 1957). It is excreted into the medium by actively photosynthesizing *Chlorella* and requires for its formation light, air, CO_2, and a medium with a pH above 5.5 (Tolbert and Zill, 1956). In the presence of glycolic acid oxidase inhibitor, it accumulates in tobacco leaves to account for over half of the total carbon fixed (Zelitch, 1959).

Both photophosphorylation (reaction 1) and the reduction of TPN (reaction 2) are indifferent to the presence of manganese (tables 2, 3), which is consistent with the scheme presented above.

The proposed scheme provides a rational basis for the experimental findings. Actual reaction mechanisms are undoubtedly more complex and numerous possible variations await further research. Reaction 4 may be substantially coupled with the oxidation of glycolic acid to glycolaldehyde, with some glycolic accumulating from side reactions. If H_2O_2 is available, as an intermediate from reaction 2 or from some aerobic process, it could react reductively with the Mn^{+++} compound in lieu of reaction 4. Alternatively, H_2O_2 could form a percarbonate and reaction 3 would produce an Mn^{+++} perhydroxyl compound which would decompose forming oxygen and divalent manganese.

LITERATURE CITED

Arnon, E. I. 1958. Chloroplasts and Photosynthesis pp. 181–233, The Photochemical Apparatus. The Brookhaven National Laboratory. 366 p.

———. 1959. Conversion of light into chemical energy in photosynthesis. Nature 184: 10–21.

Bassham, J. A. and **M. Calvin.** 1957. The Path of Carbon in Photosynthesis. Prentice-Hall, Inc. 104 p.

Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and **W. Stepka.** 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J. Amer. Chem. Soc. 72: 1710–18.

Brown, T. E., H. C. Eyster and **H. A. Tanner.** 1958. Physiological Effects of Manganese Deficiency, pp. 135–156. Trace Elements. Academic Press, Inc. 410 p.

Cayle, T. and **R. Emerson.** 1957. Effect of wave length on the distribution of carbon-14 in the early products of photosynthesis. Nature 179: 89–90.

Eyster, H. C., T. E. Brown and **H. A. Tanner.** 1958. Mineral Requirements for *Chlorella pyrenoidosa* under Autotrophic and Heterotrophic Conditions. pp. 157–174. Trace Elements. Academic Press, Inc. 410 p.

Treharne, R. W., T. E. Brown, H. C. Eyster and **H. A. Tanner.** 1960. Electron Spin Resonance Studies of Manganese in *Chlorella pyrenoidosa*. Biochem. Biophys. Res. Commun. In press.

Warburg, O. and **G. Krippahl.** 1958. Hill Reactions. Zeitft. Naturforsch. 13B: 509–14.

Zelitsch, I. 1959. The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves. J. Biol. Chem. 234: 3077–3081.