A MANGANESE DEPENDENT PHOTOSYNTHETIC PROCESS

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Manganese deficient plants which have a negligible Hill reaction activity still produce photosynthetic oxygen at a substantial fraction of the rate for manganese normal plants (Brown *et al*, 1958). This suggests that two metabolic paths exist, one of which requires manganese and is responsible for the Hill reaction.

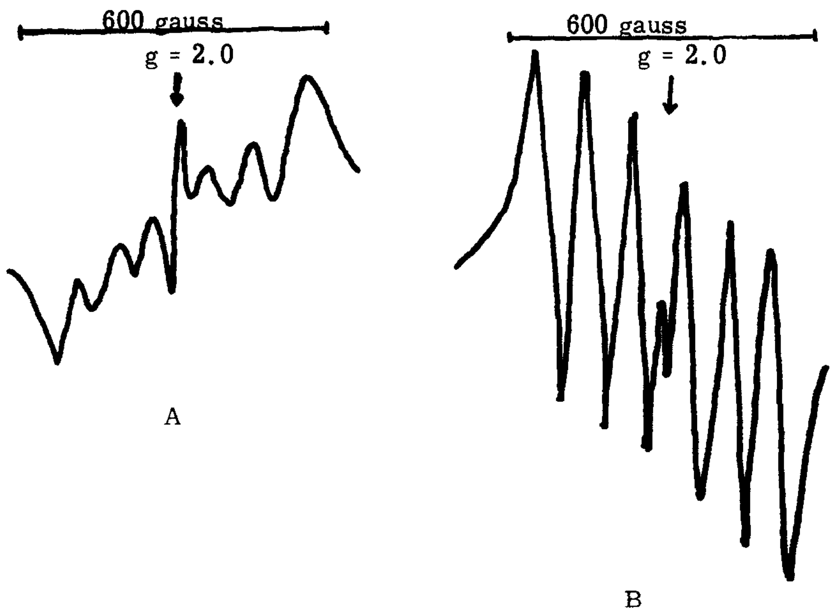

Figure 1. Electron Spin Resonance Signals. Chlorella pyrenoidosa cells with normal manganese content. Varian Model 4500 EPR Spectrometer, 100 Kc. modulation. Samples illuminated by 200 watt tungsten bulb with 0.25% $CuCl_2$ solution filtering. A-Pretreated by one hour illumination with access to air. B-Pretreated by one hour illumination in closed vessel to reduce available CO_2.

Chlorella cells in visible light exhibit an electron spin resonance (ESR) free radical signal which only appears in the presence of manganese, not in manganese deficient cells. The Mn^{2+} ESR signal decreases slowly in the light and recovers in the dark (Treharne et al, 1960). A CO_2 requirement for the appearance of the light and manganese dependent free radical has now been demonstrated. CO_2 was depleted in a culture by holding it in the light for one hour in a closed vessel. After this treatment the free radical signal is reduced and the Mn^{2+} signal is quite strong (Figure 1).

These results indicate that CO_2 is involved in the formation of a free radical signal with the concurrent oxidation or complexing of Mn^{2+}.

Conventional chromatographic and radio autographic procedures (Benson et al, 1950) were employed to investigate the effect of manganese on the distribution of C^{14} labeled photosynthetic products in Chlorella. The results (Table 1) reveal a pronounced manganese dependent accumulation of glycolic acid. Glycolic acid is readily oxidized to glyoxylic acid (Tolbert and Cohan, 1953) and those other intermediates, such as malic and succinic acids, which are more heavily labeled in the presence of manganese, could be derived from glycolic acid by this or other routes.

The labeled products from $1\text{-}C^{14}$ glycolate (Table 2) include three, alanine, serine, and sucrose, which are heavily labeled in the light compared to the dark experiment,. and the presence of manganese further increases the label in these compounds. In the case of $1\text{-}C^{14}$ glyoxylate (Table 3), alanine, serine, and sucrose are labeled prominently regardless of light or manganese suggesting that light and manganese promote the oxidation of glycolate to glyoxylate.

The results are consistent with the reduction of CO_2 to a free radical precursor of glycolic acid and the concurrent oxidation of manganese:

$$TPNH + H^{+} + CO_2 + Mn(OH)_2 \longrightarrow TPN^{+} + [HCO] + Mn(OH)_3 \qquad (1)$$

$$2\,[HCO] + H_2O \longrightarrow CH_2OHCOOH \qquad (2)$$

Table 1

Labeled Products Formed in the Light from $C^{14}O_2$ by Normal (+Mn) and Deficient (-Mn) Chlorella Pyrenoidosa (Counts per Min. per Mg. Chlorophyll) (3500 f.c. White Fluorescent Light)

	Normal (+Mn)		Deficient (-Mn)	
	10 min.	1 hr.	10 min.	1 hr.
Alanine	150,300	662,200	175,500	399,500
Aspartate	354,700	836,100	187,900	280,900
Glutamate	343,500	872,700	113,500	336,900
Glycine	2,600	73,700	10,000	128,500
Proline	-	5,200	1,200	12,600
Serine	123,800	236,400	31,600	186,600
Threonine	4,900	73,600	6,000	102,000
Tyrosine	3,400	65,900	2,800	15,400
Valine	4,300	66,800	2,400	47,300
Fumarate	19,000	30,700	10,800	5,900
Glycerate	2,300	11,800	-	6,900
Glycolate	7,200	213,000	-	6,800
Iso-citrate	11,200	26,000	36,000	11,800
Malate	52,400	149,900	2,800	23,000
Succinate	57,000	72,000	2,400	38,500
Sucrose	481,000	1,282,500	292,000	616,000
Phos. I	155,400	285,000	7,600	183,000
Phos. II	11,400	24,900	96,400	71,300

and with oxidation to glyoxylic acid at another site with the reduction of manganese:

$$4H^{+} + 2Mn(OH)_3 + CH_2OH\,COOH \longrightarrow CHOCOOH + 2Mn^{2+} + 6H_2O \qquad (3)$$

The reduction and oxidation reactions are not very directly coupled, since glycolic acid does accumulate, and they probably occur at sites where the pH is different (Tanner et al, 1960.) The reactions as written are diagrammatic and do not include all of the steps or compounds which may

Table 2

Labeled Products Synthesized from 1-C^{14} Glycolate by Normal (+Mn) and Deficient (-Mn) Chlorella Pyrenoidosa. (Counts per Min. per Mg. Chlorophyll) (3500 f.c. White Fluorescent Light)

	Light (2 hrs.)		Dark (2 hrs.)	
	-Mn	+Mn	-Mn	+Mn
Alanine	3,160	5,519	612	563
Aspartate	469	896	295	463
Glutamate	3,012	7,358	3,268	8,986
Glutathione	889	991	131	138
Serine	1,506	3,679	361	638
Unident.	370	-	230	-
Glycolate	395	1,745	3,880	1,977
Iso-citrate	617	519	1,061	188
Malate	840	943	383	463
Succinate	123	377	142	275
Sucrose	3,012	10,708	-	-

actually be involved.

The size of the glycolate pool would depend on the rate of synthesis (reaction 1) and the rate of oxidation (reaction 2). Since the latter requires two products from the former it is of a higher order with respect to CO_2 concentration. This makes possible a maximum glycolate pool at a specific CO_2 concentration which agrees with recently published experiments on the relation of glycolic acid to CO_2 pressure (Warburg et al, 1960a) and with the effect of $C^{14}O_2$ pressure on labeled glycolic acid (Wilson et al, 1955).

The close relationship of CO_2 to manganese indicated above may also explain the stimulating effect of CO_2 on the Hill reaction which has been reported (Warburg et al, 1960b).

Table 3

Labeled Products Synthesized from 1-C^{14} Glyoxylate by Normal (+Mn) and Deficient (-Mn) Chlorella Pyrenoidosa. (Counts per Min. per Mg. Chlorophyll) (3500 f. c. White Fluorescent Light)

	Light				Dark			
	-Mn	-Mn	+Mn	+Mn	-Mn	-Mn	+Mn	+Mn
	10 min.	2 hr.	10 min.	2 hr.	10 min.	2 hr.	10 min.	2 hr.
Alanine	3, 485	8, 646	4, 182	5, 211	5, 016	3, 099	4, 583	6, 461
Aspartate	155	396	386	475	258	230	513	429
Glutamate	3, 907	9, 250	5, 247	2, 599	3, 163	3, 478	4, 808	3, 673
Glutathione	1, 887	4, 146	802	3, 193	1, 773	1, 773	1, 282	2, 802
Glycine	788	-	1, 821	515	1, 086	1, 231	1, 667	670
Serine	9, 378	6, 396	10, 741	3, 522	13, 530	10, 460	14, 247	9, 263
Threonine	111	188	-	185	-	108	-	-
Iso-citrate	111	-	-	-	160	189	-	-
Malate	255	458	386	356	399	298	321	295
Succinate	44	83	93	66	128	41	144	94
Sucrose	8, 546	21, 563	15, 525	7, 757	6, 054	7, 470	6, 587	5, 952
Hexose di-P.	133	354	201	237	160	14	112	161
Triose-P.	111	833	185	594	48	122	-	172

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