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THE EFFECT OF HIGH PARTIAL PRESSURES OF OXYGEN ON PHOTOSYNTHESIS IN *CHLORELLA*—I.

THE EFFECT ON END PRODUCTS OF PHOTOSYNTHESIS

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Abstract—During periods of photosynthesis in $^{14}CO_2$ of up to 70 min at concentrations of carbon dioxide up to 4 per cent, the major end products were sucrose and a polyglucan. At the lower concentrations of carbon dioxide the addition of isonicotinyl hydrazide (INH) stimulated the production of glycollate and glycine but did not affect the total fixation. Raising the partial pressure of oxygen or the addition of iodoacetamide increased the production of glycollate but decreased the total fixation. At higher concentrations of carbon dioxide both the inhibition of photosynthesis by oxygen and the stimulation in production of glycollate were decreased; whereas the inhibition of photosynthesis by iodoacetamide increased. Carbon-14 was incorporated into the sugar phosphates of the photosynthetic cycle during short term photosynthesis and the further metabolism of this carbon studied during photosynthesis in $^{12}CO_2$. The main radioactive products were sucrose and polyglucan. At low concentrations of carbon dioxide, raising the partial pressure of oxygen or the addition of INH led to an accumulation of glycollate. Similar effects of high partial pressure of oxygen and addition of INH were found on the products of photometabolism of exogenous [U-^{14}C]glucose in carbon dioxide free conditions.

INTRODUCTION

IN *Chlorella* most of the carbon dioxide fixed during photosynthesis is incorporated ultimately into carbohydrates (sucrose and polyglucan) and lipid compounds. Carbon dioxide is incorporated into fructose-1,6-diphosphate by means of the carboxydismutase reaction and the photosynthetic carbon reduction cycle[1]. It may be further metabolized to a variety of carbohydrates by the reactions shown in Fig. 1.

However, at low concentrations of carbon dioxide, glycollic acid becomes a significant product of photosynthesis;[2, 3] most of the glycollate produced is excreted into the external medium.[4] Radioactive glycollate is also formed by *Chlorella* when fed with radioactive glucose in the light.[5]

The production of glycollate from the oxidation of glycoaldehyde-thiamine pyrophosphate transketolase addition complex has been demonstrated by Bradbeer and Racker[6] and by

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da Fonseca-Wollheim *et al.*[7] Bassham and Kirk[8] have proposed that the mechanism of the carboxydismutase reaction involves the intermediate formation of a similar complex between a thiazol grouping on the enzyme (similar to thiamine pyrophosphate) to give a phosphoglycoaldehyde addition compound. A similar oxidation of such a C-2 complex would lead to the formation of phosphoglycollate. Richardson and Tolbert[9] have shown that very active phosphatases are present in plant tissues which are specific for phosphoglycollate.

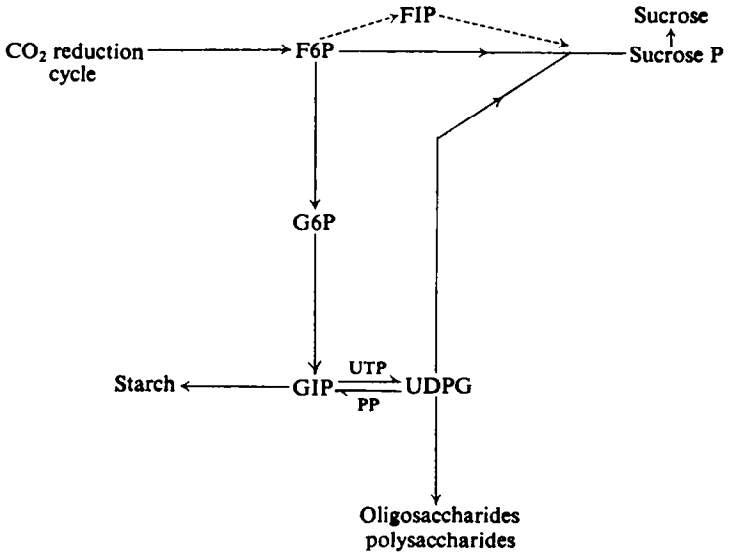

FIG. 1. BIOSYNTHETIC PATHWAYS FOR SYNTHESIS OF CARBOHYDRATES.

The further metabolism of glycollate via glyoxylate, glycine and serine to triose has been termed the C-2 or glycollate pathway. Interconversion of intermediates of this pathway has been demonstrated in animal tissues[10–12] and leaves of higher plants.[13–17]

Whittingham *et al.*[18–20] have shown that addition of isonicotinyl hydrazine (INH) to *Chlorella* increases glycine and glycollate formation with a corresponding decrease in serine formation. These results are consistent with the inhibition of the conversion of glycine to serine and would suggest that INH should inhibit the C-2 pathway.

The production of glycollate during photosynthesis is greatly increased when the partial

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pressure of oxygen is increased.[21–23] Higher partial pressure of oxygen can inhibit a number of enzyme reactions in which —SH groups are important; iodoacetamide (IOD) is also known to complex with such groups.[24] Both high partial pressure of oxygen[25] and iodoacetamide[26] inhibit photosynthesis. The present investigation compares the effects of high partial pressure of oxygen and the addition of iodoacetamide or isonicotinyl hydrazide on the total fixation of carbon-14 and the production of glycollic acid in photosynthesis.

RESULTS

Cell suspensions from 3–4 day cultures of *Chlorella pyrenoidosa* (Emerson strain) were illuminated in a Perspex lollipop,[19] flushed with air or oxygen for 30 min, and sodium carbonate-^{14}C solution injected at a constant rate into the base of the lollipop whilst the suspension was bubbled with the requisite gas stream (e.g. see Table 1). Samples were killed in boiling 80% ethanol, further extracted with 20% ethanol, and the combined extracts analysed by the procedure of Benson *et al.*[27] The method of introduction of ^{14}C to the *Chlorella* by

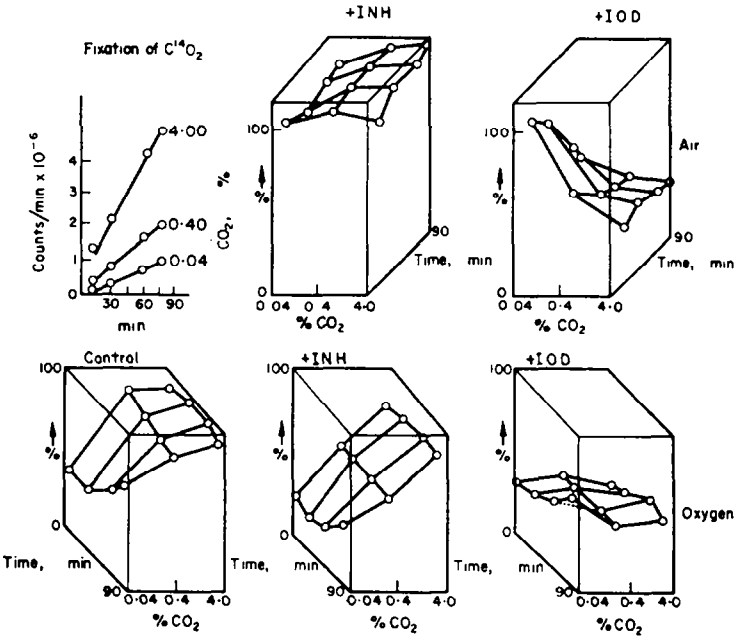

FIG. 2. THE FIXATION OF RADIOACTIVE CARBON DIOXIDE IN PHOTOSYNTHESIS AS A FUNCTION OF TIME UNDER VARIOUS CONDITIONS AND AT DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE.
Each value is shown as a percentage of the corresponding rate in air without inhibitor.

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TABLE 1. THE EFFECT OF CARBON DIOXIDE AND OXYGEN CONCENTRATION ON THE PERCENTAGE INCORPORATION OF ^{14}C INTO END PRODUCTS OF PHOTOSYNTHESIS IN THE PRESENCE OR ABSENCE OF INH OR IOD.

Carbon dioxide (%)	Gas stream	Inhibitor†	^{14}C in given end product* Ethanol insoluble (%)	Sucrose (%)	Glycollate (%)	Lipid (%)
0·04	Air	None	60	15	2	15
		INH	48	6	27	9
		IOD	36	30	8	8
	Oxygen	None	37	17	32	5
		INH	40	5	39	5
		IOD	24	16	40	6
0·40	Air	None	68	12	1	11
		INH	62	8	5	12
		IOD	62	17	2	8
	Oxygen	None	65	10	4	8
		INH	60	8	15	7
		IOD	60	11	14	8
4·00	Air	None	70	10	1	10
		INH	72	12	1	7
		IOD	70	13	1	8
	Oxygen	None	74	10	1	7
		INH	68	8	1	6
		IOD	76	10	1	6

* After 1 hr photosynthesis. † INH 10^{-2} M; IOD 10^{-5} M.

continuous injection into the base of the lollipop was shown to give a steady rate of incorporation throughout the experimental period (Fig. 2). The fixation with the various treatments has been expressed as a percentage of that in 20 per cent oxygen and the appropriate carbon dioxide concentration. The addition of INH caused a slight increase in fixation of the order of 20 per cent at 4 per cent carbon dioxide in air. At the lowest carbon dioxide concentration iodoacetamide had only a slight inhibitory effect after 15 min photosynthesis in $^{14}CO_2$ (45 min in the inhibitor), but the degree of inhibition increased with time. At higher carbon dioxide concentrations the inhibition was greater. In contrast the inhibition induced by raising the partial pressure of oxygen was not time dependent and was most marked at lower partial pressures of carbon dioxide.

In Table 1 the incorporation into the chief end products of photosynthesis is expressed as a percentage of the total fixation at that carbon dioxide concentration after one hour photosynthesis in $^{14}CO_2$. Over 90 per cent of the total carbon fixed was found in the following four fractions: ethanol insolubles, sucrose, glycollate and lipids.

The composition of the ethanol insoluble fraction was investigated by acid hydrolysis. The main component was glucose, which accounted for 80 to 90 per cent of the incorporated radioactivity. Where the incorporation of activity into this fraction was inhibited the

decrease could be accounted for by the decrease in incorporation into the glucose resulting from hydrolysis.

In air (controls) the percentage incorporation of ^{14}C into the ethanol insoluble fraction was not affected by carbon dioxide concentration. It was decreased by addition of INH, IOD, or by raising the oxygen partial pressure but the decrease was less at the higher concentration of carbon dioxide.

In air the production of glycollate was negligible even at low concentrations of carbon dioxide; under the same conditions the addition of INH or an increase in oxygen partial pressure resulted in a marked increase in the incorporation of carbon-14 into glycollate. Addition of IOD also resulted in a small increase in glycollate formation. These effects

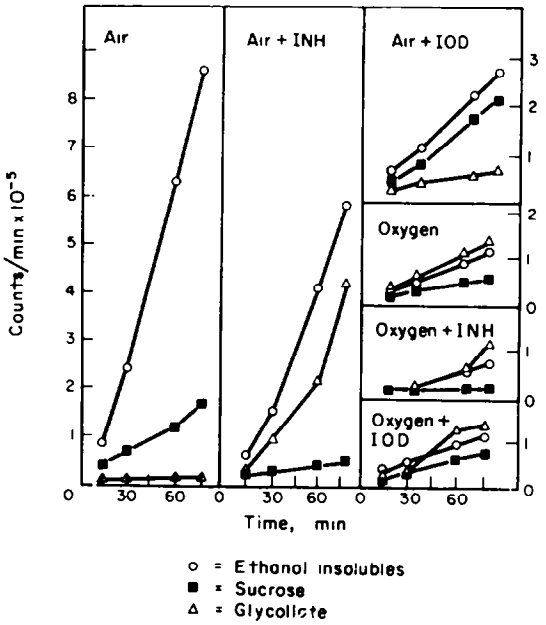

FIG. 3. TIME COURSE OF INCORPORATION OF RADIOACTIVITY FROM CARBON DIOXIDE INTO END PRODUCTS OF PHOTOSYNTHESIS IN THE PRESENCE OF 0·04 PER CENT CARBON DIOXIDE.

decreased as the carbon dioxide concentration was increased and at the highest concentration of carbon dioxide little incorporation of carbon-14 into glycollate was observed with any treatment.

The percentage incorporation into sucrose was also not affected by the various treatments at high concentrations of carbon dioxide. As the carbon dioxide concentration was decreased the sucrose became more sensitive to the addition of INH.

At low concentrations of carbon dioxide the percentage incorporation into lipids was inhibited by both INH and IOD and by high partial pressure of oxygen. As the carbon dioxide concentration was raised these effects became less marked.

The above results do not take into account the differences in total fixation with the different treatments. The time course of incorporation of activity into the end products at the lowest concentration of carbon dioxide is shown in Fig. 3. These results indicate that the absolute amount of glycollate formed in the presence of INH in air is two or three times that formed in the presence of high oxygen partial pressure. Although iodoacetamide inhibited both the

TABLE 2. THE EFFECT OF VARIOUS TREATMENTS, IN THE ABSENCE OF $^{14}CO_2$, ON THE METABOLISM OF ^{14}C INCORPORATED INTO THE SUGAR PHOSPHATES (AFTER 20 MIN)

				Glycollate		Amino acids		
	Lipid (counts/min +10⁻³)	Polyglucan (counts/min ×10⁻³)	Sucrose (counts/min ×10⁻³)	External (counts/min ×10⁻³)	Internal (counts/min ×10⁻³)	Glycine (counts/min ×10⁻³)	Serine (counts/min ×10⁻³)	Alanine, glutamate and aspartate (counts/min +10⁻³)
*Series 1**								
0·04% CO_2								
Air	9·6	70·0	3·9	4·0	3·4	2·3	4·0	9·2
Air+INH	9·7	36·0	4·2	38·0	4·3	21·2	1·9	1·8
Oxygen	18·1	37·0	2·5	30·0	7·5	2·3	2·8	4·2
Oxygen+INH	10·3	30·0	3·1	30·0	8·1	11·8	0·7	7·1
Carbon dioxide free								
Air	13·1	64·5	5·1	15·0	3·5	4·0	5·8	5·8
Air+INH	9·3	36·0	4·6	41·0	2·3	23·6	1·7	2·0
Oxygen	7·1	33·0	5·4	31·0	6·5	5·0	3·6	5·1
Oxygen+INH	6·2	26·0	1·7	50·0	8·7	13·2	0·8	1·4
Series 2							Unspecified amino acids	
0·04% CO_2								
Air light	13·0	53·0	15·3	3·0			14·0	
Air dark	11·0	44·0	6·0	2·0			26·0†	
Oxygen light	17·0	30·0	11·0	16·0			12·5	
Oxygen dark	19·0	45·0	5·0	6·0			25·3†	
4·0% CO_2								
Air light	10·0	51·0	20·0	tr			7·0	
Air dark	13·0	49·0	18·0	tr			19·0†	
Oxygen light	11·0	50·0	12·0	tr			9·0	
Oxygen dark	15·0	44·0	13·0	tr			25·0†	

* INH, 10^{-2} M. † Mainly alanine.

total fixation and the incorporation into ethanol insolubles, the incorporation of ^{14}C into sucrose was slightly higher than that in the air control.

In further experiments to investigate the effect of oxygen and carbon dioxide concentration on photosynthesis the cells were preincubated in air (containing 0·04 per cent $^{12}CO_2$) the gas stream was then stopped and sodium carbonate-^{14}C injected into the lollipop. After 1 min a sample was taken. It was found that about 50 per cent of the radioactivity was incorporated into sugar phosphates. The suspension was then flushed with a rapid stream of air or oxygen containing only $^{12}CO_2$, in either light or dark. A further sample was then taken after 20 min. In some cases the sample was divided and one part filtered, and glycollate determined in the filtrate. It was found that the residual ^{14}C was removed from the suspension in a few seconds so that little further fixation was detectable. Hence the further metabolism of carbon which had been incorporated into the sugar phosphate could be investigated (Table 2).

In the first experiments (series 1) the formation of glycollate in the air control was low and the major product was polyglucan. In the second experiments (series 2) the production of glycollate in the air control was also low, but more sucrose was formed at the expense of polyglucan. These differences indicate the variation between different cell cultures.

At lower concentrations of CO_2 glycollate formation was enhanced by high oxygen partial pressure and INH. There was a corresponding decrease in incorporation into the polyglucan fraction. These effects were enhanced when gas free of carbon dioxide was used for flushing. If the cells were incubated with 4 per cent CO_2 after fixation of $^{14}CO_2$ at air levels of carbon dioxide the main products were polyglucan and sucrose. With this concentration no glycollate appeared with any treatment. Dark treatment produced little glycollate, even at low concentrations of carbon dioxide, but mainly polyglucan and amino acids (alanine, aspartate and glutamate).

Although both addition of INH and raising the partial pressure of oxygen cause an increase in glycollate production under low carbon dioxide it is clear that the action of these factors differs. The addition of INH leads to a marked increase in glycine formation as reported by us previously,[20] but has little effect on the amount of internal glycollate, whereas high partial pressure of oxygen had little effect on the formation of glycine and led to an increase in internal glycollate. The addition of INH also decreased the incorporation of radioactivity into alanine and aspartate. This is consistent with the inhibition of transaminase reactions.

Table 3 shows the results of experiments in which the further metabolism of $^{14}CO_2$ fixed

TABLE 3. THE PERCENTAGE DISTRIBUTION OF CARBON-14 INTO END PRODUCTS OF PHOTOSYNTHESIS FROM $^{14}CO_2$ OR D-GLUCOSE-U-^{14}C METABOLIZED IN CARBON DIOXIDE FREE CONDITIONS

	$^{14}CO_2$				[U-^{14}C]Glucose			
	Air		Oxygen		Air		Oxygen	
INH*	−	+	−	+	−	+	−	+
Polyglucan	50	30	31	33	40	36	28	21
Glycollate	4	21	26	34	7	25	23	29
Sucrose	23	16	14	9	15	9	14	7
Lipid	8	9	11	6	14	14	10	17

* 10^{-2} M.

into the sugar phosphates of the photosynthetic cycle allowed to run through to end products of photosynthesis in the light without internal carbon dioxide, is compared with the photometabolism of glucose-U-^{14}C under the same conditions. The similarity in the effects of raising the partial pressure of oxygen or addition of INH on the production of glycollate suggests that glucose may be incorporated into intermediates of the photosynthetic cycle before conversion to glycollate. Whether radioactivity is supplied either from carbon dioxide or from glucose, the ^{14}C which in the air control was incorporated into polyglucan or sucrose appears in the presence of INH as glycollate. However the degree of inhibition of polyglucan by INH was greater from carbon dioxide than from glucose. The converse was true for sucrose.

DISCUSSION

The results indicate that at low concentrations of carbon dioxide the incorporation of radioactivity into glycollate is increased when INH is added or the oxygen partial pressure is raised and that the incorporation into carbohydrates decreases correspondingly. This is found to be the case whether the fixation of $^{14}CO_2$ or photometabolism of glucose is investigated. Although addition of INH or raising the partial pressure of oxygen both increase the production of glycollate they differ in that INH has little effect on the rate of photosynthesis whereas oxygen markedly inhibits it. Our results do not indicate that the inhibition of photosynthesis by oxygen is similar to that by iodoacetamide.

These results are in agreement with those of Tamiya and Huzisige[28] in that raising the partial pressure of oxygen results in a greater inhibition of photosynthesis at lower concentrations of carbon dioxide. It has been suggested that the effect of oxygen on photosynthesis is due to the inhibition of an enzyme of the photosynthetic cycle such as glyceraldehyde phosphate dehydrogenase[29] through an oxidation of —SH groups on the enzyme. Iodoacetamide has been shown to be an inhibitor of reactions in which such groups are involved. However the inhibitory action of oxygen and that of IOD are shown here to differ. The inhibition of photosynthesis at low concentrations of carbon dioxide by oxygen is accompanied by an increased production of glycollic acid. Glycollate is not found at higher concentrations of carbon dioxide, and oxygen does not markedly affect the rate of photosynthesis under these conditions. However there is no necessary relationship between glycollate production and inhibition of photosynthesis since glycollate production is also enhanced by the addition of INH at low concentrations of carbon dioxide, when a slight stimulation of the rate of photosynthesis is also observed.

In oxygen the addition of INH led to the inhibition of photosynthesis even at an intermediate carbon dioxide concentration. This inhibition was again associated with an increase in glycollate production.

It is suggested that at low concentrations of carbon dioxide the photosynthetic carbon reduction cycle produces a C-2 fragment from which glycollate may be formed. Under conditions of higher partial pressure of oxygen, or when INH is added, a large amount of glycollate is excreted, leading to a decrease in the amount of carbon remaining in the photosynthetic cycle intermediates. However oxygen inhibits photosynthesis whereas INH does not. The simplest explanation for these results would suggest that C-2 fragments are normally produced from the cycle intermediates at low concentrations of carbon dioxide, and are

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further metabolized via a C-2 pathway to end products. If INH blocked the C-2 pathway without altering the rate of production of C-2 compounds from the cycle the amount of glycollate and glycine accumulating under these conditions would give an indication of the amount of carbon being metabolized via the C-2 pathway. High partial pressure of oxygen may increase the rate of oxidation of the C-2 fragments to glycollate thus increasing the rate of loss of carbon from the cycle intermediates and leading to inhibition of photosynthesis.

When glucose is fed, polyglucan is found to be inhibited less in INH than when it is formed from endogenous sugar phosphates. This would be consistent with the suggestion that part of the glucose is converted to polyglucan in the cytoplasm. At high partial pressure of oxygen the sugar phosphate pools of the chloroplast are depleted as C-2 fragments are oxidized to glycollate and under these conditions sugars may be diverted from the cytoplasm to the chloroplasts. Thus part of the exogenous glucose is converted to glycollate and consequently less appears as carbohydrate.

It is concluded that oxygen inhibits photosynthesis at low concentrations of carbon dioxide by increasing the loss of carbon from the intermediates of the cycle rather than by the specific inhibition of a particular enzyme step.

METHODS

Three to four day cultures of *Chlorella pyrenoidosa* (Emerson strain) grown on 4% carbon dioxide in air at 22° with continuous illumination. The cells were harvested by centrifugation, washed once in distilled water and resuspended in potassium dihydrogen phosphate solution (10^{-4} M; pH 4·5–5·0). A cell density of 5 μl wet packed cell volume per ml of suspension was used.

The cell suspension was illuminated in a Perspex "lollipop" as described previously,[19] but at a light intensity of 8600 L. All suspensions were first illuminated and aerated with a stream of air or oxygen for 30 min prior to any other treatment. Sodium carbonate-^{14}C in solution was continuously injected at a constant rate into the base of the lollipop whilst the suspension was bubbled with a gas stream of known carbon dioxide concentration. The amount of total carbon dioxide generated from the carbonate-^{14}C was not sufficient to significantly alter the carbon dioxide concentration.

Samples were taken at intervals and killed in boiling 80% ethanol. The cells were further extracted with 20% ethanol and the extracts combined. Subsequent analysis followed the procedure described by Benson *et al.*[27] Radioactivity was counted directly on paper chromatograms using a thin end window Geiger-Muller tube. INH was obtained from Lights Chemicals Ltd. and iodoacetamide from British Drug Houses Ltd. and were recrystallized before use. The inhibitors were added at the commencement of illumination to a final concentration of 10^{-2} M INH and 10^{-5} M IOD.

When radioactive glucose was fed it was added at a concentration of 1 μc ^{14}C (6 μg of glucose-^{14}C) per 10 ml of suspension at the end of a 30 min preincubation period. The samples were divided, one part was processed as usual, the other was filtered in the light. This separated the unused glucose and excreted glycollate which could be separately determined after chromatography.

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