

## Photosynthesis by Isolated Chloroplasts

### INHIBITION BY DL-GLYCERALDEHYDE OF CARBON DIOXIDE ASSIMILATION

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Photosynthetic carbon assimilation and associated CO<sub>2</sub>-dependent O<sub>2</sub> evolution by chloroplasts isolated from pea shoots and spinach leaves is almost completely inhibited by 10 mM-DL-glyceraldehyde. The inhibitor is without appreciable effect on photosynthetic electron transport, photophosphorylation, the carboxylation of ribulose 1,5-diphosphate or the reduction of 3-phosphoglycerate, but apparently blocks the conversion of triose phosphate into ribulose 1,5-diphosphate.

Many compounds which inhibit photosynthesis are relatively unspecific. At low concentrations (0.1 μM) 3-(3,4-dichlorophenyl)-1,1-dimethylurea is an exception, possibly attacking only one or two sites associated with photosystem II (Izawa *et al.*, 1966). Certainly its effect on photosynthetic induction phenomena observed in whole chloroplasts (Walker & Crofts, 1970) is consistent with inhibition of some photochemical event because it produces the same response as a decrease in light intensity or a decrease in chlorophyll content. Conversely an inhibition producing a specific block at some step in the Benson-Calvin cycle might be expected to exhibit kinetics similar to those caused by a decrease in temperature. During a study of the effect of several factors on induction phenomena (Baldry *et al.*, 1966) we examined a number of inhibitors of photosynthesis but in each case, except for 3-(3,4-dichlorophenyl)-1,1-dimethylurea, the kinetics of response indicated a multiplicity of effects involving both 'light' and 'dark' reactions. However, DL-glyceraldehyde came nearest to what we would have expected of a purely 'dark' inhibitor and accordingly we have sought to locate its site of action.

#### Experimental

##### *Plant material*

Peas (var. Feltham First) were grown for 8-11 days in vermiculite at 18°C, with a light-intensity of 2000 lx for 11 h/day. Spinach was grown locally in the field, or purchased from George Walker of Covent Garden, London W.C.2, U.K.

##### *Intact chloroplasts*

These were prepared as described by Walker (1971) from pea shoots or spinach leaves which had been

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pre-illuminated for 30 min at about 20000 lx. The pea shoots were cut into iced water and used immediately to avoid post-harvesting changes (which do not occur in spinach). The grinding medium for peas contained 0.33 M-sorbitol, 5.0 mM-MgCl<sub>2</sub>, sodium isoascorbate (0.2%, w/v), NaCl (0.1%, w/v), 50 mM-KH<sub>2</sub>PO<sub>4</sub> and 50 mM-Na<sub>2</sub>HPO<sub>4</sub>, adjusted to pH 6.5 with HCl. For spinach preparations, sodium pyrophosphate (10 mM) was substituted for orthophosphate. After 3 s of maceration in a Polytron blender the leaf homogenate was squeezed through two layers of muslin and filtered through eight layers of muslin enclosing one layer of cotton-wool. After centrifugation (see Walker, 1971) the chloroplasts were resuspended in an assay medium which contained 50 mM-HEPES buffer [2-(N-2-hydroxyethyl)piperazin-N'-yl]ethane sulphonic acid], 0.33 M-sorbitol, 1 mM-MgCl<sub>2</sub>, 1 mM-MnCl<sub>2</sub> and 2 mM-EDTA adjusted to pH 7.6 with KOH (Stokes & Walker, 1971b).

##### *Envelope-free chloroplasts*

Intact chloroplasts were rendered envelope-free by resuspension in 200 ml of grinding medium that had been diluted 50-fold (see Walker, 1971). A chloroplast pellet was recovered from this suspension by centrifugation and resuspended in full-strength assay medium as before. These chloroplasts were used in the experiments illustrated in Figs. 6-8.

##### *Reconstituted chloroplasts*

Whole chloroplasts were prepared from peas or spinach (as above) by using the orthophosphate medium only (Stokes & Walker, 1971b). After the first centrifugation the chloroplast pellet was resuspended in 11 ml of assay medium that had been diluted 25-fold. After the second centrifugation the supernatant was retained and designated 'chloroplast extract' and the osmotically shocked chloroplasts were resuspended in full-strength assay medium as

before. The chloroplast extract and the chloroplasts prepared in this fashion were used together as a 'reconstituted' system in experiments illustrated in Figs. 10–12. Unlike envelope-free chloroplasts, the reconstituted system will evolve  $O_2$  with  $HCO_3^-$  or 3-phosphoglycerate as substrates because the addition of chloroplast extract, ferredoxin,  $NADP^+$  etc. offsets the dilution of soluble components (approx. 1/500–1/100 in a mixture containing  $100\mu g$  chlorophyll/ml) which normally follows rupture of the chloroplast envelope (Stokes & Walker, 1971*a,b*; Walker *et al.*, 1971).

### $CO_2$ fixation

For assay of 'dark'  $CO_2$  fixation (Fig. 9) intact chloroplasts were osmotically shocked in the reaction vessel to permit entry of ATP and ribulose 1,5-diphosphate which are believed not to penetrate the intact envelope (Walker, 1969; Stokes & Walker, 1971*a*; Walker & Crofts, 1970). This was achieved by varying the addition of reactants (water before double-strength assay medium) so that for approx. 60s the chloroplasts were exposed to hypo-osmotic conditions which causes them to swell and burst (Walker, 1969, 1971; Walker & Crofts, 1970). Reactions were started by the further addition of ribulose 1,5-diphosphate, ribose 5-phosphate or ribulose 5-phosphate to mixtures to which  $Na_2^{14}CO_3$  had just been added (for further details of reaction mixtures see legends to Fig. 9). Small samples were then removed at intervals, acidified, dried, plated and counted for radioactivity as previously described (e.g. Bucke *et al.*, 1966; Walker & Hill, 1967). A similar method of sampling, counting etc., was employed for the light-dependent  $CO_2$  fixation shown in Fig. 3.

### $O_2$ evolution

$O_2$  was measured (see Delieu & Walker, 1972) simultaneously in twin cells fitted with Clark-type electrodes and illuminated at  $20^\circ C$  with near-saturating red light provided by quartz-iodine slide projectors (150W, 24V) fitted with heat filters and a broad red filter transmitting light above 600 nm.

### Biochemicals

Sugar phosphates, coenzymes etc. were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Ferredoxin was kindly given by Dr. D. O. Hall.

## Results

### Experiments with intact chloroplasts

Figs. 1 and 2 show that at a concentration of 10mM, DL-glyceraldehyde completely inhibited  $O_2$  evolution by intact isolated chloroplasts when these were

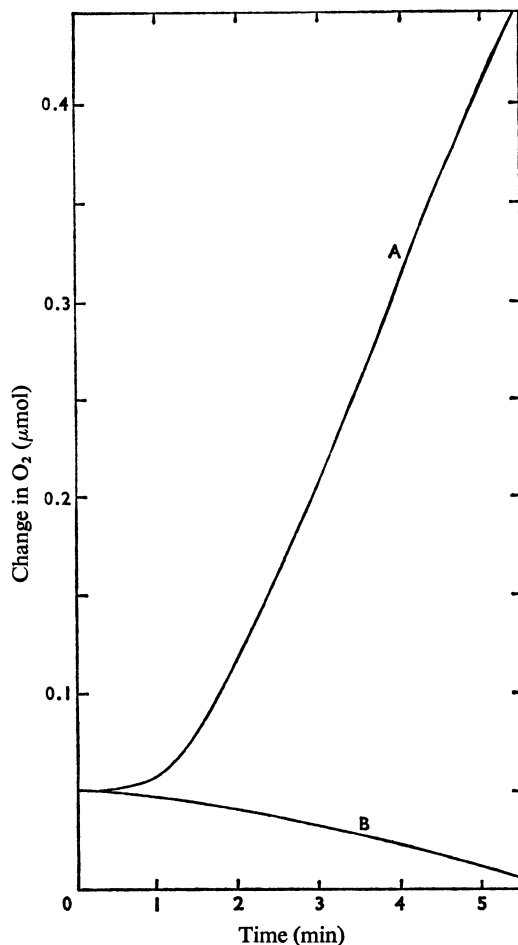


Fig. 1. Inhibition by glyceraldehyde of  $CO_2$ -dependent  $O_2$  evolution by intact chloroplasts

Both reaction mixtures contained chloroplasts ( $100\mu g$  of chlorophyll) and  $NaHCO_3$  ( $20\mu mol$ ) in 2ml of assay medium. In addition one reaction mixture (curve B) contained 10mM-DL-glyceraldehyde.

provided with  $CO_2$  as the sole added substrate. The inhibition was reversed by the subsequent addition of 3-phosphoglycerate (Fig. 2) but not by ribose 5-phosphate (Fig. 2), nor by ribulose 5-phosphate nor fructose 6-phosphate (not shown). The restoration of  $O_2$  evolution by 3-phosphoglycerate was not maintained and  $O_2$  evolution soon decreased to a very slow rate. Similarly, when 3-phosphoglycerate was included in a reaction mixture from the outset (Fig. 3)  $O_2$  evolution started almost immediately (cf. Walker & Hill, 1967) and continued at a fast rate, whereas if glyceraldehyde was also present the  $O_2$  evolution was initially the same but soon decreased. Fig. 3 also

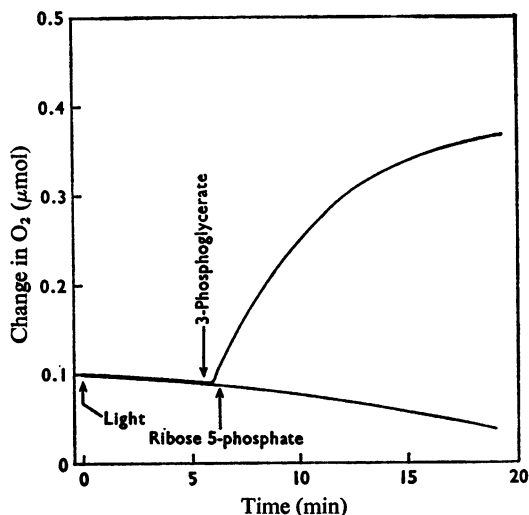


Fig. 2.  $O_2$  evolution by intact chloroplasts in the presence of DL-glyceraldehyde

Both reaction mixtures contained 10mM-DL-glyceraldehyde, 20  $\mu$ mol of  $NaHCO_3$ , 50  $\mu$ g of chlorophyll and 1  $\mu$ mol of orthophosphate. In addition 3  $\mu$ mol of 3-phosphoglycerate and 3  $\mu$ mol of ribose 5-phosphate were added as indicated. In essentially similar experiments ribulose 5-phosphate and fructose 6-phosphate were substituted for ribose 5-phosphate, with the same lack of  $O_2$  production.

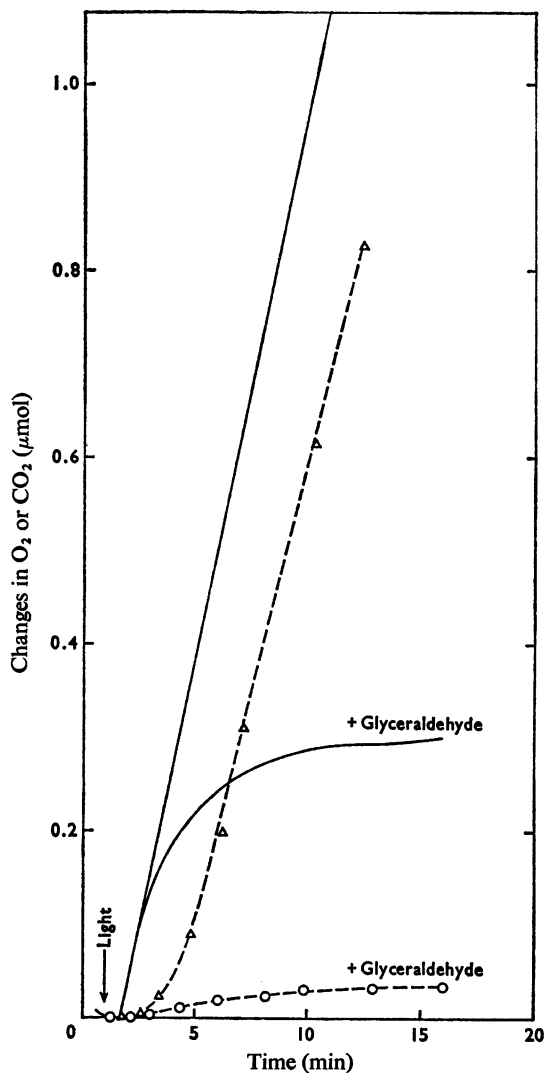


Fig. 3.  $O_2$  evolution (—) and  $CO_2$  fixation (----) by intact chloroplasts in the presence of phosphoglycerate,  $CO_2$  and glyceraldehyde

Both reaction mixtures contained chloroplasts (100  $\mu$ g of chlorophyll), 3-phosphoglycerate (3  $\mu$ mol),  $NaHCO_3$  (20  $\mu$ mol) and 100  $\mu$ Ci of  $Na_2^{14}CO_3$  (1.82  $\mu$ mol) in 2ml of assay medium. In addition one reaction mixture contained 10mM-DL-glyceraldehyde. Measurements (see the Experimental section) of  $O_2$  evolution ( $O_2$  electrode) and  $CO_2$  fixation ( $^{14}C$  sampling) were made simultaneously in the same vessel.

with the chloroplast preparation (presumably because of differences in endogenous  $CO_2$  and Benson-Calvin-cycle metabolites), but sooner (Fig. 4) or later (Fig. 5)

records the measurements of  $CO_2$  fixation made simultaneously in the same mixtures. In the absence of the inhibitor, the time course of  $CO_2$  fixation showed the characteristic initial lag (Walker & Crofts, 1970), which is also seen in  $O_2$  evolution measurements (Fig. 1 and Fig. 4, curve B) when  $CO_2$  is the sole added substrate. In the presence of glyceraldehyde,  $CO_2$  fixation is inhibited as would be expected from the inhibition of the associated  $O_2$  evolution (Fig. 1). Together, therefore, these results suggest that glyceraldehyde is a potent inhibitor of carbon assimilation (and its associated  $O_2$  evolution), but that it is initially without effect on phosphoglycerate-dependent  $O_2$  evolution. After a short time-interval, however, inhibition of phosphoglycerate-dependent  $O_2$  evolution develops and progresses until it becomes complete. This secondary progressive inhibition could be explained if phosphoglycerate-dependent  $O_2$  evolution is governed by the accumulation of one or more intermediates normally removed by the further reactions of the Benson-Calvin cycle. A similar decline in phosphoglycerate-dependent  $O_2$  evolution is observed in mixtures in which the further reactions of the cycle are limited by the omission of added  $CO_2$  (Figs. 4 and 5). The kinetics of this latter decline vary

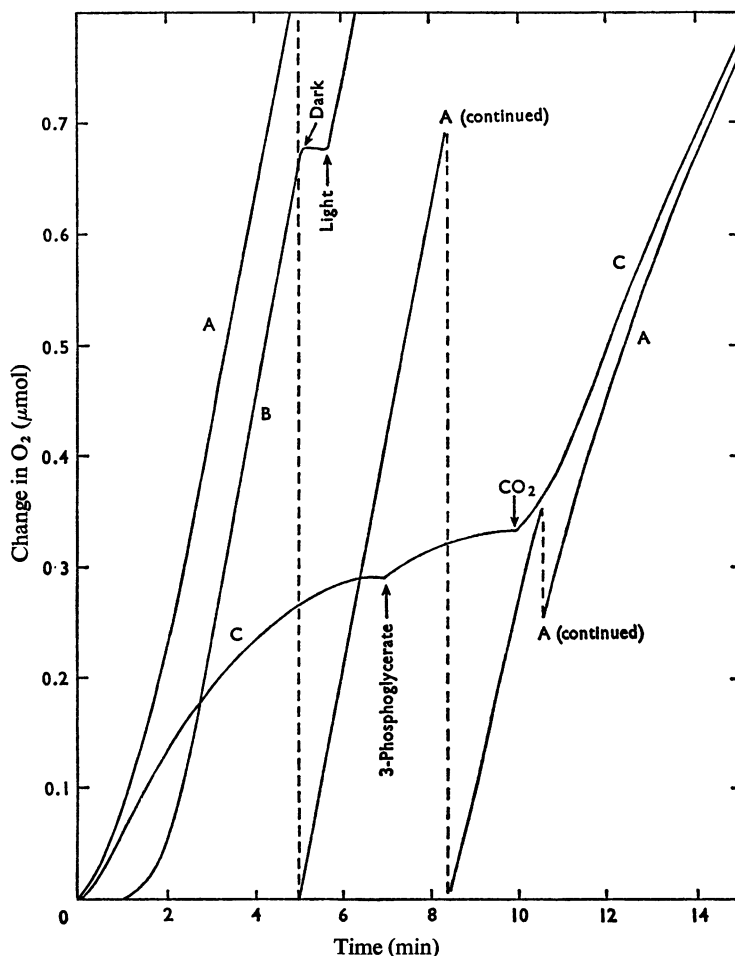


Fig. 4. Decline in phosphoglycerate-dependent  $O_2$  evolution in the absence of  $CO_2$

Each reaction mixture contained intact chloroplasts ( $100\mu g$  of chlorophyll) in 2ml of assay medium. Other materials present were: 3-phosphoglycerate ( $2\mu mol$ ) and  $NaHCO_3$  ( $20\mu mol$ ), curve A;  $NaHCO_3$  ( $20\mu mol$ ), curve B; phosphoglycerate ( $2\mu mol$ ), curve C. In the last experiment further phosphoglycerate ( $2\mu mol$ ) and  $NaHCO_3$  ( $20\mu mol$ ) were then added, as indicated. --- Represents the electrical re-setting of the pen to keep curve A on the recording chart. Curves A and C were recorded simultaneously and for B (as well as A and C) illumination was started at zero time. Curve B is included for purposes of comparison to show the kinetics observed in the absence of added phosphoglycerate (cf. Fig. 1). This lag in the attainment of maximum rate of  $O_2$  evolution corresponds to the onset of  $CO_2$  fixation (cf. Fig. 3,  $\Delta$ ).

the rate in the absence of added  $CO_2$  declines and finally  $O_2$  evolution ceases (cf. curves A and C in Fig. 4 and curves A and B in Fig. 5). At this point the further addition of phosphoglycerate produces a much smaller increment of  $O_2$  than would be anticipated if  $O_2$  evolution had ceased through lack of phosphoglycerate (cf. Fig. 10, curves A and B).

Evolution of  $O_2$  can still be reinitiated, however, by the addition of  $CO_2$  (Fig. 4, curve C; Fig. 5, curve B) and the rate then rapidly approaches that seen in a reaction mixture which has contained both  $CO_2$  and phosphoglycerate from the outset. In all of these respects reaction mixtures containing both  $CO_2$  and glyceraldehyde behave in much the same way as

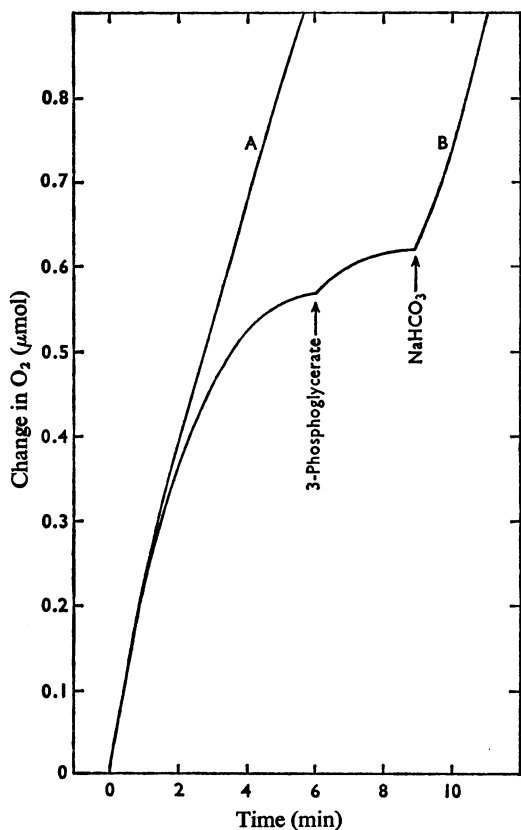


Fig. 5. Decline in phosphoglycerate-dependent  $O_2$  evolution in the absence of  $CO_2$

The experiment was done as in Fig. 4 with 3-phosphoglycerate ( $2\mu\text{mol}$ ) present from the outset (curves A and B). Also present were  $NaHCO_3$  ( $20\mu\text{mol}$ ), curve A;  $NaHCO_3$  ( $20\mu\text{mol}$ ) and phosphoglycerate ( $2\mu\text{mol}$ ) added as indicated, curve B. Although the kinetics and extent of  $O_2$  evolution (with phosphoglycerate as sole substrate) were somewhat different from those shown in Fig. 4 the decline in  $O_2$  evolution and its recovery (after the addition of  $CO_2$ ) were essentially the same in both cases. A comparison of Figs. 4 and 5 illustrates the extent to which the detailed kinetics vary from preparation to preparation. The kinetics of the decline in phosphoglycerate-dependent  $O_2$  evolution caused by lack of  $CO_2$  in Figs. 4 and 5 should be compared with that caused by the presence of glyceraldehyde in Figs. 2 and 3.

those containing neither (cf.  $O_2$  evolution in Fig. 3 with Figs. 4 and 5), again suggesting that glyceraldehyde interferes specifically with carbon assimilation.

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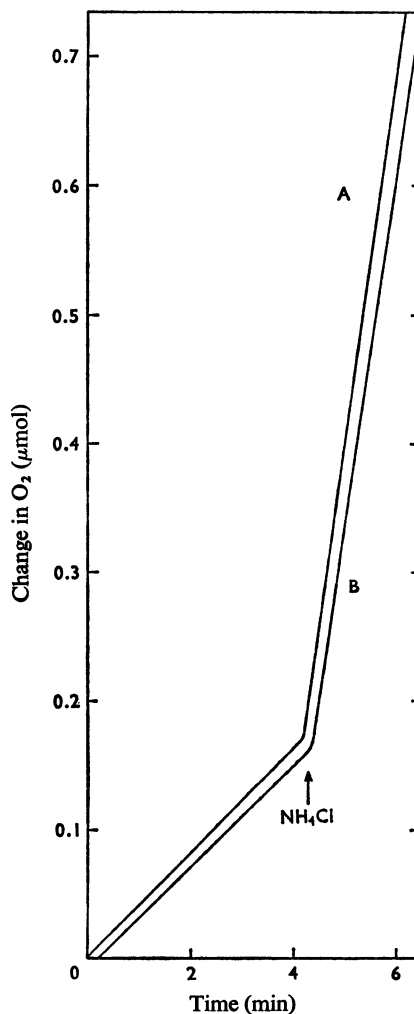


Fig. 6. Lack of detectable effect of glyceraldehyde on coupled and uncoupled electron transport with ferricyanide as Hill oxidant

In addition to the constituents of the assay medium each reaction mixture (2ml) contained chloroplasts ( $100\mu\text{g}$  of chlorophyll), orthophosphate ( $2\mu\text{mol}$ ), ADP ( $2\mu\text{mol}$ ) and potassium ferricyanide ( $3.5\mu\text{mol}$ ).  $NH_4Cl$  ( $5\mu\text{mol}$ ) was added as indicated. One reaction mixture (curve A) also contained  $10\text{mM-DL-glyceraldehyde}$ .

#### *Experiments on electron transport and photophosphorylation using envelope-free chloroplasts*

If an inhibitor affects carbon assimilation or its associated  $O_2$  evolution, it may do so by direct inhibition of some partial reaction of the Benson-Calvin

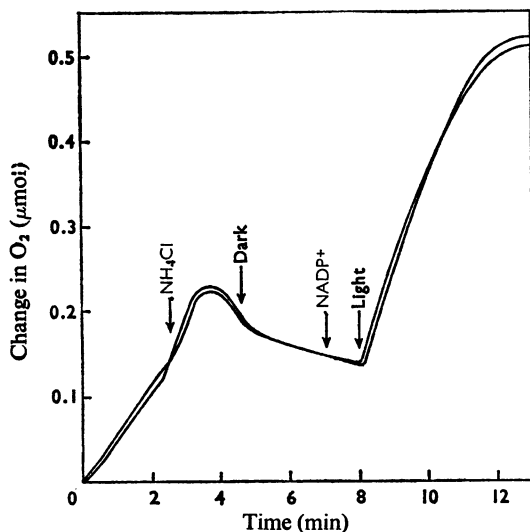


Fig. 7. Lack of appreciable effect of glyceraldehyde on coupled and uncoupled electron transport with  $\text{NADP}^+$  as Hill oxidant

The experiment was done as in Fig. 6 but  $\text{NADP}^+$  (approx.  $0.5 \mu\text{mol}$ ) and ferredoxin (approx.  $3 \text{nmol}$ ) replaced the ferricyanide. After the addition of  $\text{NH}_4\text{Cl}$  (as indicated) the accelerated  $\text{O}_2$  evolution soon ceased and changed to a Mehler reaction ( $\text{O}_2$  uptake) as the added  $\text{NADP}^+$  became fully reduced. After a brief dark interval during which more  $\text{NADP}^+$  was added (approx.  $1 \mu\text{mol}$ )  $\text{O}_2$  evolution was resumed when illumination was restarted. The presence of DL-glyceraldehyde ( $10 \text{mM}$ ) had little, if any, effect, as indicated by the closeness of the curves.

cycle. Alternatively it might act indirectly, for example by interfering with a process such as electron transport or photosynthetic phosphorylation. Fig. 6 shows that  $10 \text{mM}$ -glyceraldehyde was without effect on electron transport when ferricyanide was used as the Hill oxidant in the presence of ADP and orthophosphate. Similarly no significant difference in rate between experiment and control could be detected if ADP and phosphate were omitted or if electron transport was uncoupled by the addition of  $\text{NH}_4\text{Cl}$  (as in Fig. 6). Essentially similar results were also obtained when ferredoxin and substrate concentrations of  $\text{NADP}^+$  were substituted for ferricyanide (Fig. 7). Again glyceraldehyde did not change the rate of coupled or uncoupled electron transport, implying that photophosphorylation was also unaffected. Direct measurements of the changes in acidity associated with cyclic and non-cyclic photophosphorylation (Nishimura *et al.*, 1962; Crofts, 1968) confirmed this conclusion. For example, Fig. 8 shows

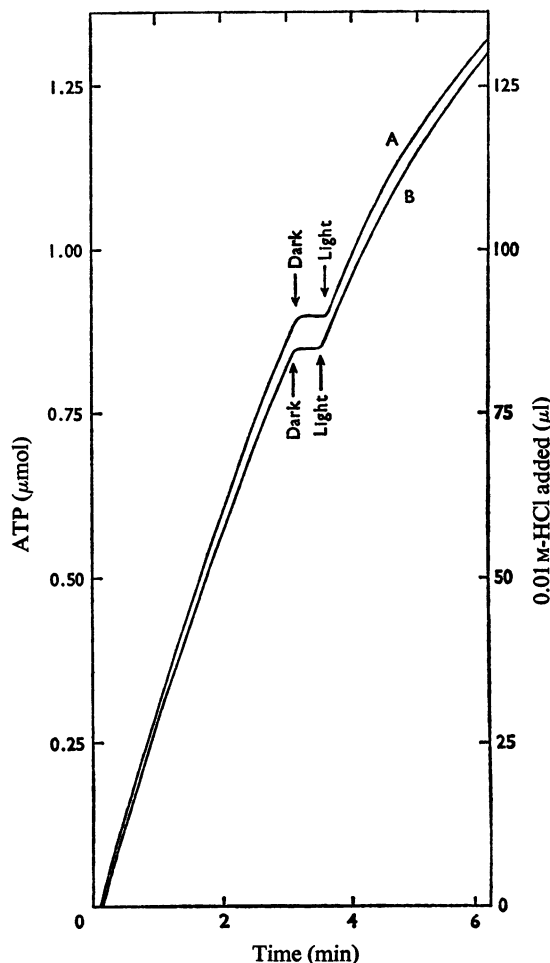


Fig. 8. Lack of detectable effect of glyceraldehyde on cyclic photophosphorylation with pyocyanine as cofactor

Each reaction mixture contained chloroplasts ( $200 \mu\text{g}$  of chlorophyll) in  $0.33 \text{M}$ -glucose- $1\%$   $\text{KCl}$ - $0.01 \text{M}$ - $\text{MgCl}_2$  together with orthophosphate ( $4 \mu\text{mol}$ ), ADP ( $4 \mu\text{mol}$ ) and pyocyanine ( $0.6 \mu\text{mol}$ ) initially adjusted to  $\text{pH} 8.0$  with  $0.01 \text{M}$ - $\text{NaOH}$  in approx.  $4 \text{ml}$ . The  $\text{pH}$  was maintained at this value during illumination by recorded automatic titration with  $0.01 \text{M}$ - $\text{HCl}$ . One reaction mixture (curve B) also contained  $10 \text{mM}$ -DL-glyceraldehyde.

that the time-course of cyclic photophosphorylation with pyocyanine as cofactor (Walker & Hill, 1958) was unchanged in the presence of  $10 \text{mM}$ -glyceraldehyde.

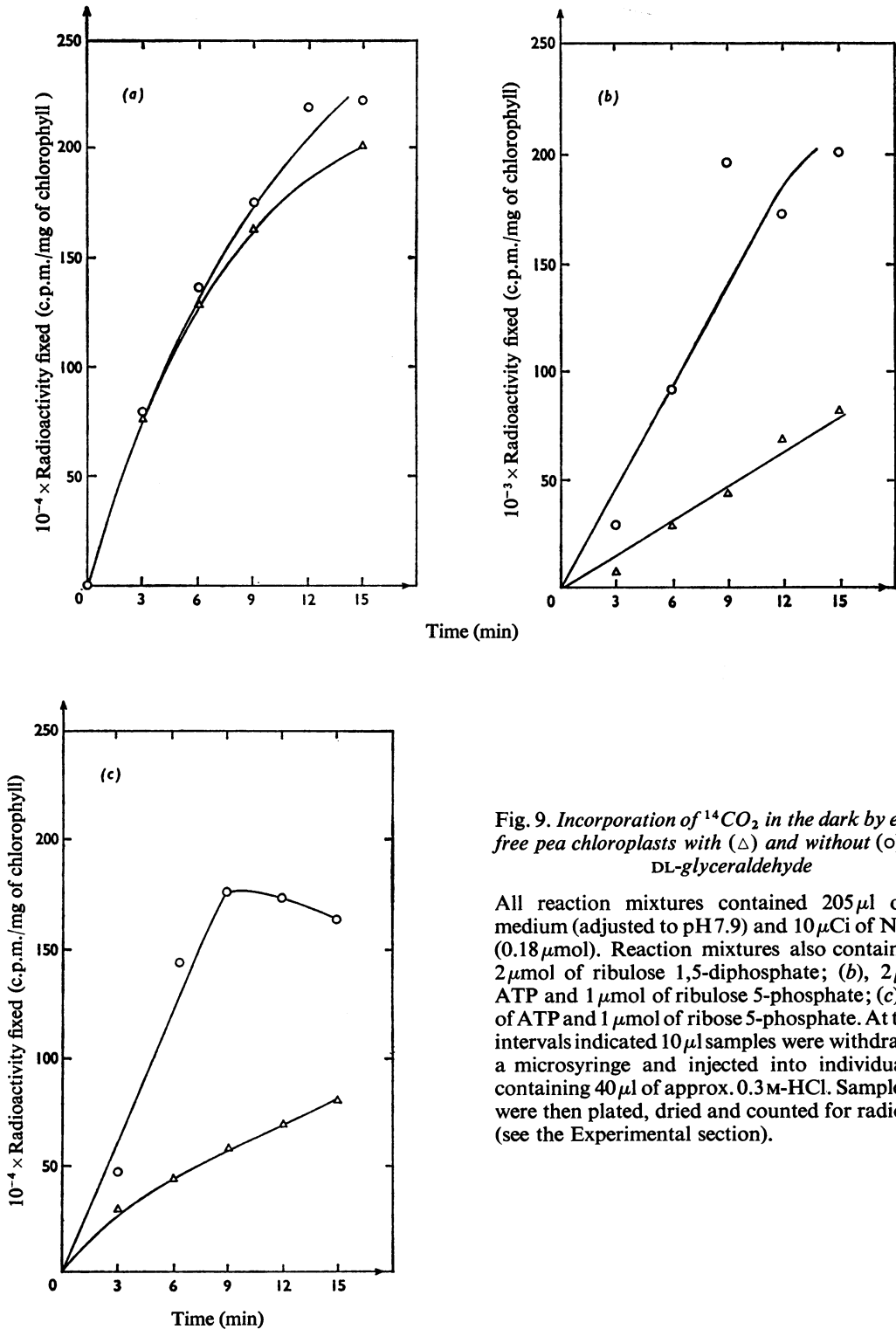


Fig. 9. Incorporation of  $^{14}\text{CO}_2$  in the dark by envelope-free pea chloroplasts with ( $\Delta$ ) and without (o) 10 mM-DL-glyceraldehyde

All reaction mixtures contained 205  $\mu\text{l}$  of assay medium (adjusted to pH 7.9) and 10  $\mu\text{Ci}$  of  $\text{Na}_2^{14}\text{CO}_3$  (0.18  $\mu\text{mol}$ ). Reaction mixtures also contained: (a), 2  $\mu\text{mol}$  of ribulose 1,5-diphosphate; (b), 2  $\mu\text{mol}$  of ATP and 1  $\mu\text{mol}$  of ribulose 5-phosphate; (c), 2  $\mu\text{mol}$  of ATP and 1  $\mu\text{mol}$  of ribose 5-phosphate. At the time-intervals indicated 10  $\mu\text{l}$  samples were withdrawn with a microsyringe and injected into individual tubes containing 40  $\mu\text{l}$  of approx. 0.3 M-HCl. Samples (20  $\mu\text{l}$ ) were then plated, dried and counted for radioactivity (see the Experimental section).

*Experiments on CO<sub>2</sub> fixation using envelope-free chloroplasts*

The above results pointed to a possible effect of glyceraldehyde on the primary photosynthetic carboxylation or on some step between triose phosphate and the substrate for this carboxylation. Accordingly, experiments were done to determine the effect of glyceraldehyde on CO<sub>2</sub> fixation with ribulose 1,5-diphosphate as substrate. In the experiments outlined in the preceding section envelope-free chloroplasts were preferred because the intact envelope is known to offer a barrier to ferricyanide, NADP<sup>+</sup> and ADP. Similarly, to achieve 'dark' CO<sub>2</sub> fixation it is necessary to use envelope-free chloroplasts to allow access of added ATP and ribulose 1,5-

diphosphate (Walker, 1969; Walker & Crofts, 1970; Stokes & Walker, 1971a). Since osmotic shock is normally followed by release of soluble enzymes into the supernatant during centrifugation, the chloroplasts required for CO<sub>2</sub> fixation were ruptured within the reaction mixture (see the Experimental section). Fig. 9(a) shows that with ribulose 1,5-diphosphate as substrate there was no significant inhibition of CO<sub>2</sub> fixation by 10mM-glyceraldehyde. However, with either ribose 5-phosphate (Fig. 9b) or ribulose 5-phosphate (Fig. 9c), in mixtures containing added ATP to permit the preliminary formation of ribulose 1,5-diphosphate, there was an appreciable inhibition, which was similar with both substrates, suggesting that there must be some effect of glyceraldehyde on

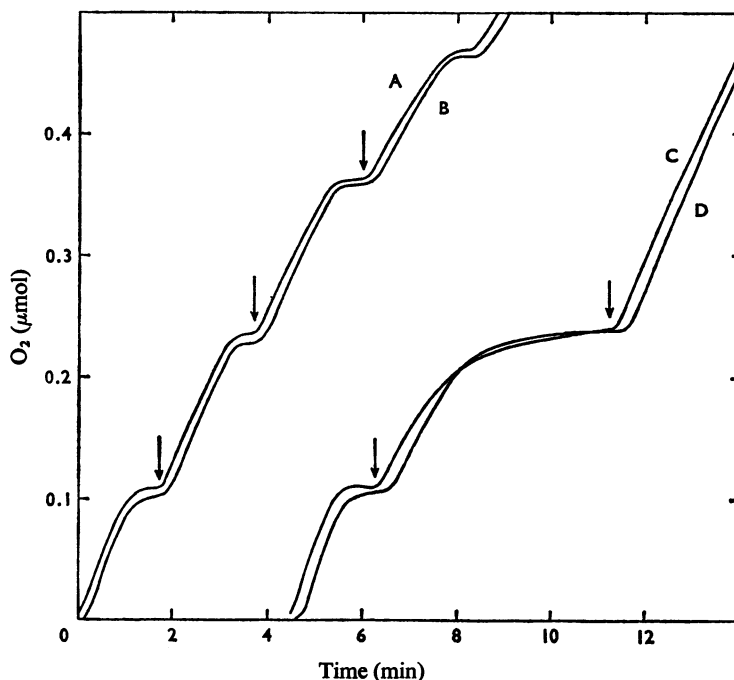


Fig. 10. *Lack of detectable effect of glyceraldehyde on O<sub>2</sub> evolution associated with the conversion of ribulose diphosphate into triose phosphate*

Each 2 ml of reaction mixture contained chloroplast extract (0.8 ml), envelope-free chloroplasts (100  $\mu$ g of chlorophyll), double-strength assay medium (1.0 ml), additional MgCl<sub>2</sub> (to give a final concentration of 5 mM), ferredoxin (approx. 3 nmol), NADP<sup>+</sup> (approx. 0.2  $\mu$ mol), ATP (4  $\mu$ mol) and NaHCO<sub>3</sub> (10  $\mu$ mol). The chloroplast extract was obtained (see the Experimental section) by osmotically shocking intact chloroplasts (approx. 10 mg of chlorophyll) in 11 ml of 1/25-strength assay medium and contained approx. 12 mg of protein/ml. The DL-glyceraldehyde was added (curves B and D) to a final concentration of 10 mM. In curves A and B illumination was begun simultaneously at zero time; after the first cessation of O<sub>2</sub> evolution (caused by reduction of all the added NADP<sup>+</sup>), 3-phosphoglycerate (0.4  $\mu$ mol in 4  $\mu$ l) was added three times to each as indicated. In curves C and D illumination was also begun simultaneously at zero time, but the traces have been displaced to the right by 4.5 min for convenience. After the first cessation of O<sub>2</sub> evolution 0.2  $\mu$ mol of ribulose 1,5-diphosphate was added to each. After O<sub>2</sub> evolution had again ceased it was restarted by adding an excess of phosphoglycerate (2  $\mu$ mol).



the step immediately leading to the formation of ribulose 1,5-diphosphate (ribulose 5-phosphate kinase).

#### Experiments with a reconstituted chloroplast system

Osmotically shocked chloroplasts lose the ability to evolve  $O_2$  with 3-phosphoglycerate or  $CO_2$  as substrates (Walker & Crofts, 1970) but recently such  $O_2$  evolution has been demonstrated in a 'reconstituted' system containing envelope-free chloroplasts, chloroplast extract plus additional  $NADP^+$  and ferredoxin (Stokes & Walker, 1971*b*; Walker *et al.*, 1971). By using the reconstituted system it was possible to confirm that 10mM-glyceraldehyde had no effect on  $O_2$  evolution associated with the reduction of added  $NADP^+$  and 3-phosphoglycerate. Thus the initial  $O_2$  evolution seen in Figs. 10–12 is that associated with the reduction of endogenous and added  $NADP^+$  (cf. Fig. 7) and ceases in the absence of subsequent additions. If 3-phosphoglycerate is then added (as in Fig. 10, curves A and B)  $O_2$  evolution is resumed (as reduced  $NADP^+$  is reoxidized in triose phosphate formation) and there was no effect of glyceraldehyde either on the rate or the extent of phosphoglycerate-dependent  $O_2$  evolution (cf. Fig. 10, curves A and B). In view of this result and the failure of glyceraldehyde to inhibit  $^{14}CO_2$  fixation with ribulose 1,5-diphosphate as substrate (Fig. 9*a*), it was anticipated that ribulose diphosphate-dependent  $O_2$  evolution would be similarly unaffected and this prediction was borne out by the experiment also illustrated in Fig. 10 (curves C and D). In this experiment (as in others illustrated here) the  $O_2$  electrode traces were recorded simultaneously in pairs by using twin vessels (see Delieu & Walker, 1972). [It is of interest that the stoichiometry observed with ribulose 1,5-diphosphate, i.e.  $O_2$  evolved/molecule of substrate added, was consistent with the conversion of one molecule of ribulose 1,5-diphosphate into two molecules of 3-phosphoglycerate and tallied with the manufacturers evaluation of the ribulose diphosphate content of their product (cf. Fig. 10, curves A and B with Fig. 10, curves C and D). The stoichiometry between added phosphoglycerate and evolved  $O_2$  in Fig. 10 is consistent with a steady-state value (under these conditions) of 60–70% conversion of 3-phosphoglycerate into triose phosphate.]

Surprisingly, in view of the results shown in Figs. 9(*b*) and 9(*c*), there was initially no effect of glyceraldehyde on the rate of  $O_2$  evolution when ribose 5-phosphate in relative excess ( $2\mu\text{mol}$ ) was substituted for ribulose 1,5-diphosphate. As the concentration of ribose 5-phosphate was lowered (to  $0.8\mu\text{mol}/2\text{ml}$ ), however, the extent of  $O_2$  evolution was diminished (Fig. 1, curves A and B) and at still lower concentrations ( $0.4\mu\text{mol}/2\text{ml}$ ) both extent and maximum rate of evolution were affected (Fig. 12, curves A and B).

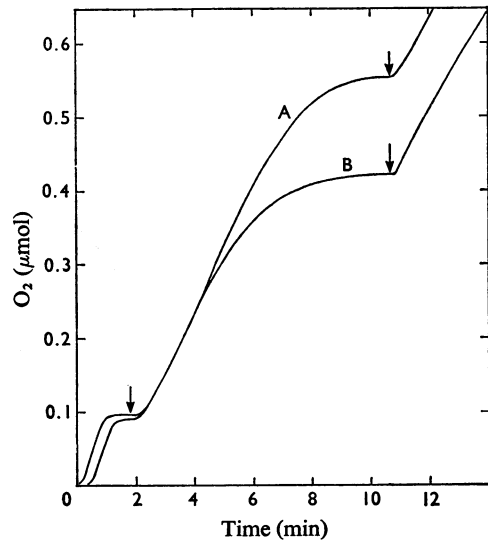


Fig. 11. Depression by glyceraldehyde of the extent of  $O_2$  evolution initiated by ribose 5-phosphate

The experiment was done as in Fig. 10 with 10mM-glyceraldehyde present (curve B) but ribose 5-phosphate ( $0.8\mu\text{mol}$ ) was added (both curves) after the first cessation of  $O_2$  evolution. Finally an excess of phosphoglycerate ( $2\mu\text{mol}$ ) was added (both curves) after the second cessation of  $O_2$  evolution.

Essentially similar inhibitions were observed when ribulose 5-phosphate (Fig. 12, curves C and D) or xylulose 5-phosphate (not shown) were used instead of ribose 5-phosphate. In all of these experiments  $O_2$  evolution was fully restored by the subsequent addition of  $2\mu\text{mol}$  of phosphoglycerate. (Direct quantitative comparison of Fig. 9 with Figs. 10–12 is not possible because of the presence of chloroplast extract in the experiments shown in Figs. 10–12.) Osmotically shocked chloroplasts, as in Fig. 9, have no ability to evolve  $O_2$ , at rates that can be measured at present, with either  $CO_2$  or phosphoglycerate as substrates, partly at least, because of the dilution of soluble components which are normally retained by the intact envelope. In the reconstituted system this ability is restored by the addition of such components to a working concentration (see the Experimental section).

#### Discussion

We have shown that DL-glyceraldehyde inhibits photosynthetic carbon assimilation in intact chloroplasts, but that it appears to be entirely without effect on the many partial reactions of electron transport and photophosphorylation or on the carboxylation

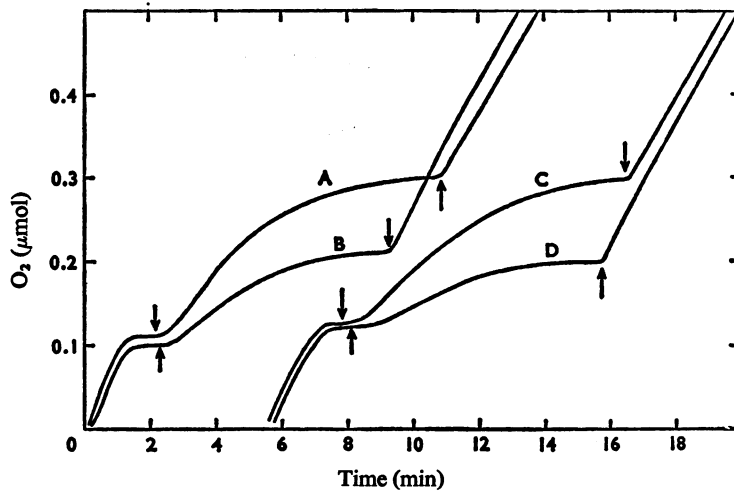


Fig. 12. Depression by glyceraldehyde of the extent and initial rate of  $O_2$  evolution initiated by lower concentrations of pentose monophosphates

The experiment was done as in Fig. 11 with 10mM-glyceraldehyde present (curves B and D). The traces were recorded simultaneously in pairs with illumination at zero time, but curves C and D are displaced to the right by 5.5 min for convenience. After the first cessation of  $O_2$  evolution, ribose 5-phosphate ( $0.4 \mu\text{mol}$ ) was added (curves A and B). For curves C and D ribulose 5-phosphate ( $0.4 \mu\text{mol}$ ) was added. Finally,  $2 \mu\text{mol}$  of 3-phosphoglycerate was added (all curves). Essentially similar results were obtained when xylulose 5-phosphate was added in place of ribose 5-phosphate.

of ribulose 1,5-diphosphate and the subsequent reduction of 3-phosphoglycerate to triose phosphate. This implies that it must interfere in some way with one or more steps in the conversion of triose phosphate into ribulose 1,5-diphosphate and the experiments with ruptured chloroplasts (Fig. 9) and the reconstituted system (Figs. 10–12) indicate that there is substantial inhibition of the formation of ribulose 1,5-diphosphate from ribulose 5-phosphate. The progressive nature of this latter effect, seen most clearly in Figs. 11 and 12, is of interest in two respects. First, such kinetics might be expected if glyceraldehyde brings about its effects by diverting part of the normal photosynthetic carbon traffic into abnormal products. Secondly, this could contribute to the fact that 10mM-glyceraldehyde inhibited carbon assimilation in intact chloroplasts completely but that, initially at least, it only caused a partial inhibition of the  $CO_2$  fixation (Fig. 9b and c) and  $O_2$  evolution (Figs. 11 and 12) sustained by pentose monophosphates in experiments with envelope-free chloroplasts and the reconstituted system respectively. Clearly in these circumstances the actual degrees of inhibition observed would depend on the concentration of pentose monophosphate added to the envelope-free chloroplasts and the reconstituted system on the one hand, and on the steady-state concentration of pentose mono-

phosphates normally maintained within the intact chloroplasts on the other. However, it is by no means certain that only one partial reaction between triose phosphate and pentose diphosphate is affected and if glyceraldehyde does act by diversion of carbon flow rather than as an enzyme inhibitor, there are at least four plausible sites of action (see below) and such multiplicity of sites would also contribute to the different degree of response referred to above. Similarly if glyceraldehyde, however surprisingly, acted as a competitive inhibitor of an enzyme such as ribulose 5-phosphate kinase then neither the different degrees of inhibition observed in the several systems nor the progressive nature of the inhibition in Figs. 11 and 12 would be entirely unexpected or inexplicable.

Glyceraldehyde is known to serve as an alternative condensation partner with dihydroxyacetone phosphate in the reaction catalysed by aldolase (Tung *et al.*, 1954) and if this reaction occurred in the chloroplast, it would divert carbon into fructose 1-phosphate and sorbose 1-phosphate rather than fructose 1,6-diphosphate and (subsequently) fructose 6-phosphate. Glyceraldehyde can also act as an acceptor for  $C_2$  units in the transketolase reaction (Horecker *et al.*, 1953; de la Haba *et al.*, 1955) and could conceivably drain carbon (as free xylulose and ribulose) from fructose 6-phosphate,

sedoheptulose 7-phosphate and xylulose 5-phosphate. Our results do not allow us to distinguish between these various possibilities (although there is evidently little likelihood that the condensation of dihydroxyacetone phosphate and glyceraldehyde is directly implicated in the inhibition shown in Figs. 9(b), 9(c), 11 and 12).

It remains to be resolved whether glyceraldehyde inhibits only the conversion of ribulose 5-phosphate into ribulose 1,5-diphosphate, and if it does, whether it acts directly as an inhibitor of ribulose 5-phosphate, or indirectly by draining off thiamine pyrophosphate glycoaldehyde via xylulose 5-phosphate and a transketolase reaction to yield free xylulose and ribulose. Indeed, if glyceraldehyde does produce its effect by diverting carbon 'traffic' the inhibition might be even more subtle, with some abnormal product (or normal product at an abnormal concentration) causing an inhibition. What does seem certain at this stage is that DL-glyceraldehyde is a potent, relatively specific, and therefore potentially useful, inhibitor of photosynthesis and that it acts only by retarding the regeneration of the primary CO<sub>2</sub> acceptor from triose phosphate. In this sense, and in that it is seemingly without effect on the photochemical events in photosynthesis, it could be regarded as the 'dark' equivalent of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (see the introduction), which is believed to act only at a limited number of sites associated with photosystem II and to be entirely without direct effect on carbon assimilation.

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