Phytochemistry, 1969, Vol. 8, pp. 697 to 706. Pergamon Press. Printed in England

LIGHT-INDUCED CHANGES IN THE CONTENT OF SOME ENZYMES OF THE C_4-DICARBOXYLIC ACID PATHWAY OF PHOTOSYNTHESIS AND ITS EFFECT ON OTHER CHARACTERISTICS OF PHOTOSYNTHESIS*

M. D. HATCH, C. R. SLACK and T. A. BULL

David North Plant Research Centre, Colonial Sugar Refining Co. Ltd., P.O. Box 68, Toowong, Queensland, Australia

(*Received* 8 *October* 1968)

Abstract—Various effects of light and light-intensity on the content of several photosynthetic enzymes were examined. Included amongst the enzymes were, pyruvate, P_i dikinase, phosphopyruvate carboxylase, adenylate kinase and alkaline pyrophosphatase, enzymes believed to have specific roles in the C_4-dicarboxylic acid pathway of photosynthesis. Maize and *Amaranthus* plants were grown at high or low light intensities for prolonged periods and their enzyme contents examined before and after transfer of high-light-grown plants to low light or vice versa. Ribulose 1,5-diphosphate carboxylase activity was unaffected by these treatments, but the contents of glyceraldehyde 3-phosphate dehydrogenase, adenylate kinase and pyrophosphatase were approximately 2 fold higher and pyruvate, P_i dikinase and phosphopyruvate carboxylase between 5- and 10-fold higher in plants grown at the higher light intensities. During the 6 days following changes in the light regime the enzyme contents adapted almost completely to the new light conditions. These changes in enzyme activities were accompanied by changes in maximum photosynthesis rates and light-saturation characteristics of leaves. However studies with $^{14}CO_2$ provided no evidence for significant changes in the pathway of photosynthesis operative in these leaves. Other studies showed that the content of these and other photosynthetic enzymes were low in the leaves of maize plants germinated and grown in the dark but increased up to 15-fold, together with chlorophyll, following transfer of plants to the light for 35 hr. The possible regulatory significance of these findings are discussed.

INTRODUCTION

THE C_4-dicarboxylic acid pathway of photosynthesis is operative in several species of tropical grasses[1,2] and in species from four other families[3] including three dicotyledonous families. In addition to their characteristic labelling patterns[1–3] and enzyme activities[3–5] these plants have a unique leaf anatomy,[3,6] a different light response curve for photosynthesis and higher maximum photosynthesis rates,[3,7,8] a lower CO_2-compensation point[9] and a different response to lowered oxygen tensions,[10] compared with plants in which the Calvin Cycle is operative.

* Supported in part by a grant for equipment from the Australian Research Grants Committee.

[1] M. D. HATCH and C. R. SLACK, *Biochem. J.* **101**, 103 (1966).
[2] M. D. HATCH, C. R. SLACK and H. S. JOHNSON, *Biochem. J.* **102**, 417 (1967).
[3] H. S. JOHNSON and M. D. HATCH, *Phytochem.* **7**, 375 (1968).
[4] C. R. SLACK and M. D. HATCH, *Biochem. J.* **103**, 660 (1967).
[5] M. D. HATCH and C. R. SLACK, *Biochem. J.* **106**, 141 (1968).
[6] H. PRAT, *Ann. Sci. Nat. Botan. Ser.* **10**, 18, 165 (1936).
[7] J. HESKETH and D. N. MOSS, *Crop Sci.* **3**, 107 (1963).
[8] J. HESKETH and D. BAKER, *Crop Sci.* **7**, 285 (1967).
[9] W. J. A. DOWNTON and E. B. TREGUNNA, *Can. J. Botany* **46**, 207 (1967).
[10] R. W. DOWNES and J. D. HESKETH, *Planta* **78**, 79 (1968).

Two enzymes which are exclusive to this pathway of photosynthesis are phosphopyruvate carboxylase[4] and pyruvate, P_i dikinase,[5] the latter being previously named phosphopyruvate synthase. We have also suggested[5] that adenylate kinase and an alkaline pyrophosphatase have a specific function in this pathway by effecting the utilization of AMP and pyrophosphate respectively, both being products of the reaction catalysed by pyruvate, P_i dikinase.

The present studies provide evidence that these enzymes, together with other photosynthetic enzymes and chlorophyll, are synthesized when dark-grown maize plants are transferred to the light. Furthermore the content of these and other photosynthetic enzymes in light-grown plants was shown to adapt in response to changes of the light-intensity conditions. The effect of these changes on other characteristics of photosynthesis was examined. These results are discussed in terms of possible mechanisms for regulation of photosynthesis.

RESULTS

Comparative Activites of Adenylate Kinase and Pyrophosphatase in Leaves

Table 1 shows the activity of these enzymes in leaves of several species in which the operation of either the C_4-dicarboxylic acid pathway or Calvin pathway was previously established.[2,3] Adenylate kinase and pyrophosphatase activities were found to be higher by at least 35- and 5-fold, respectively, in species with the C_4-dicarboxylic acid pathway. The activities of the enzymes were more than adequate to account for the maximum photosynthesis rates of between 3 and 5 μmoles CO_2/min/mg chlorophyll, previously recorded for these plants.[3,4] Under the conditions of assay for pyrophosphatase the activity of non-specific phosphatases was very low.

TABLE 1. LEAF ADENYLATE KINASE AND PYROPHOSPHATASE ACTIVITIES OF PLANTS WITH THE C_4-DICARBOXYLIC ACID PATHWAY AND CALVIN PATHWAY OF PHOTOSYNTHESIS*

Photosynthesis pathway	Enzyme activity (μmoles/min/mg chlorophyll)	
	Adenylate kinase	Pyrophosphatase
C_4-dicarboxylic acid	45–17	60–15
Calvin	0·5–0·3	3·8–2·4

* Enzymes were extracted and assayed by the procedures described in the Methods section. All plants were grown in temperature-controlled greenhouses in sunlight. Some of the values for pyrophosphatase activity were taken from the previously unpublished data of Andrews and Hatch. The C_4-dicarboxylic acid pathway plants used were sugarcane, maize, *Amaranthus* and *Atriplex* and the Calvin Cycle plants were wheat, silver-beet and bean for adenylate kinase and wheat, oat, sunflower and silver-beet for pyrophosphatase. The maxima and minima for the range of different plants are given.

Effect of Light-intensity on Enzyme Activities

The ribulose 1,5-diphosphate carboxylase activity was similar in the leaves of maize and *Amaranthus* plants grown at high or low light intensities for 7 weeks (Table 2). However there was approximately twice as much glyceraldehyde 3-phosphate dehydrogenase, adenylate

TABLE 2. EFFECT OF LIGHT INTENSITY AND CHANGES OF LIGHT INTENSITY ON THE ACTIVITY OF PHOTOSYNTHETIC ENZYMES*

Light regime	Enzyme activity (μmoles/min/mg chlorophyll) at 0, 3 and 6 days																	
	Pyruvate, P_i dikinase			Phosphopyruvate carboxylase			Ribulose 1,5-P carboxylase			Glyceraldehyde 3-P dehydrogenase			Adenylate kinase			Pyrophosphatase		
	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6	0	3	6
	Amaranthus																	
High	3·6	3·1	3·5	17·0	15·0	14·5	1·3	1·1	1·3	23	19	18	70	67	54	52	49	51
Low	0·40	0·42	0·40	1·4	1·4	1·4	1·1	1·4	1·4	10	10	8	26	30	24	21	27	25
High to low	3·6	1·64	0·60	17·0	8·8	4·8	1·3	1·4	1·4	23	14	16	70	49	21	52	46	36
Low to high	0·40	1·5	2·0	1·4	11·5	14·6	1·1	1·6	1·3	10	16	12	26	49	50	21	38	40
	Maize																	
High	2·8	2·9	3·0	20	19	18	0·8	1·1	0·7	25	23	21	53	54	64	46	46	52
Low	0·52	0·57	0·46	4·7	4·0	3·8	1·1	1·3	0·9	13	11	12	30	30	27	28	33	29
High to low	2·8	1·08	0·88	20	8·4	5·9	0·8	1·0	0·9	25	12	13	53	34	35	46	34	41
Low to high	0·52	—	3·2	4·7	—	14·9	1·1	—	1·0	13	—	16	30	—	49	28	—	57

* Maize and *Amaranthus* plants were grown with a 12-hr light period at either "High" (sunlight, approx. 130,000 lux) or "Low" (8600 lux) light intensity for 7 weeks. Other conditions and procedures are described in the Methods section. All analysis were conducted on leaves which were fully expanded at day zero, the time when transfers to different light intensities occurred. For the analyses at the three times 0, 3 and 6 days, leaves were routinely collected and extracted at approx. 11 a.m. "High" and "Low" controls remained at their original light regime during the 6-day period.

kinase and pyrophosphatase in the leaves of plants grown at the higher light intensity. Even larger differences of the order of 5- to 12-fold were observed for pyruvate, P_i dikinase, and phosphopyruvate carboxylase. When plants were kept at their original light-intensity during the subsequent 6-day period these differences were maintained. However, following the transfer of plants from either high or low, or low to high light intensities at day zero the activities of pyruvate, P_i dikinase and phosphopyruvate carboxylase changed during the next 6 days to become almost comparable to the activities in the control plants grown continuously under the same light conditions (Table 2). The time required for these changes to approach completion varied both with the enzyme, and whether an increase or a decrease was involved. However other studies revealed only small changes after periods of less than 2 days.

Similar trends were observed with glyceraldehyde 3-phosphate dehydrogenase, adenylate kinase and pyrophosphatase, following changes in the light regime, but no changes were observed with ribulose 1,5-diphosphate carboxylase. It should be emphasized that the leaves used for all analyses were fully-expanded at day zero and that the treatments gave no significant changes in the chlorophyll content of leaves.

We have shown in separate studies[11, 12] that pyruvate, P_i dikinase in both maize and *Amaranthus* leaves is rapidly inactivated when plants are transferred from light to the dark and just as rapidly reactivated upon re-illumination. The process involved is quite separate from that presently being investigated. Changes in activity of more than 20-fold occur and are complete within 45–60 min. As reported elsewhere[12] the inactivated enzyme can be totally reactivated following the appropriate additions to cell-free extracts. The values for the activity of this enzyme reported in the present studies represent total potential activities since precautions were taken to reactivate any inactive enzyme which may have been present in the leaves.

Adaptations of Photosynthesis by Attached Leaves

When plants were kept at low light intensity for 6 days following growth at a high light intensity the photosynthesis characteristics of attached leaves were also altered (Fig. 1). With maize the maximum photosynthesis rate was reduced to less than half, and the optimal light-intensity for CO_2 fixation was about half of that required for control plants maintained continuously at high light intensity. Less pronounced effects were obtained with *Amaranthus*.

Pathway of Photosynthesis in Plants Adapted to Low Light Intensity

The changes in enzyme activity accompanying the reduction of the light intensity used for growth prompted an examination of the labelling patterns of similar leaves exposed to $^{14}CO_2$. If the ribulose 1,5-diphosphate carboxylase contributes to CO_2 fixation in C_4-dicarboxylic acid pathway plants, a supposition which we have questioned,[2, 4] then such a contribution should be more evident with plants adapted to low-light conditions. The time-course of labelling of photosynthetic products by leaves which had been treated for 6 days at low light intensity is shown in Table 3. No obvious differences were observed between these results and those obtained previously[1–3] for plants grown in sunlight. Neither the percentages of radioactivity in 3-phosphoglycerate and dicarboxylic acids in leaves exposed to $^{14}CO_2$ for the shortest period nor the subsequent trends in labelling provided any indication of a greater relative contribution by ribulose 1,5-diphosphate carboxylase.

[11] C. R. SLACK, *Biochem. Biophys. Res. Commun.* **30**, 483 (1968).
[12] M. D. HATCH and C. R. SLACK, *Biochem. J.*, in press (1969).

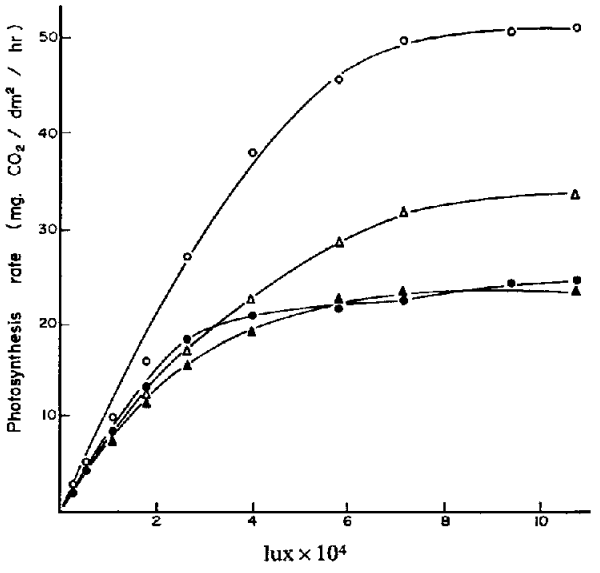

FIG. 1. EFFECT OF LOWERED LIGHT INTENSITY FOR GROWTH ON THE MAXIMUM PHOTOSYNTHESIS RATES AND LIGHT-SATURATION CHARACTERISTICS OF MAIZE AND *Amaranthus*.

The treatments (see Table 2) were maize (○) and *Amaranthus* (△) grown at "High" light and maize (●) and *Amaranthus* (▲) transferred from "High" to "Low" light 6 days prior to analysis. With the units used sunlight would be equivalent to approximately 130,000 lux.

TABLE 3. TIME-COURSE LABELLING PATTERNS FOR LEAVES OF MAIZE AND *Amaranthus* AFTER GROWTH AT REDUCED LIGHT-INTENSITY*

Time in $^{14}CO_2$ (sec)	Percentage of total ^{14}C in individual compounds			
	Malate plus asparate	3-Phospho-glycerate	Sugar phosphates	Sucrose
	Maize			
5	88	10	1	0
15	65	24	10	1
30	58	24	15	4
60	36	25	20	12
	Amaranthus			
5	87	12	1	0
15	67	17	5	0
30	62	25	12	1
60	43	23	19	13

* Analyses were made 6 days after plants grown at "High" light were transferred to "Low" light conditions. The plants used were the same as those used for the studies described in Table 2. Sugar phosphates include hexose phosphates, dihydroxyacetone phosphate and ribulose phosphates.

Table 4. Changes in the activity of photosynthetic enzymes following the transfer of dark-grown maize plants to the light

Treatment*	Chlorophyll mg/g fresh weight	Enzyme activities relative to dark control†							
		RDP carboxylase	G-3-P dehydrogenase	Pyruvate, P_i dikinase	PEP carboxylase	Adenylate kinase	Pyrophosphatase	Malate dehydrogenase	Acid phosphatase
Dark control	0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
After 16 hr light	0·7	1·9	2·3	4·5	3·8	3·5	5·0	0·7	0·8
After 35 hr light	1·3	3·2	5·3	8·5	12·7	6·2	4·9	1·0	1·1
Light control	1·5	3·6	9·1	10·0	15·0	8·1	7·0	0·9	1·0

* Maize plants were germinated and grown in complete darkness for 7 days at 28°. During the next 2 days samples of plants were transferred to a light regime consisting of sunlight between 8 a.m. and 6 p.m. and artificial light of 11,840 lux for the remaining period to give periods of 16 hr and 35 hr illumination. For all analyses leaves were removed at 11 a.m. on the 9th day. Dark controls remained in the dark for the full period. Light controls were plants germinated at the same time but grown at 28° in the greenhouse in sunlight between 8 a.m. and 6 p.m. All analyses were made on primary leaves which were fully-expanded by 7 days.

† Results were calculated initially as μmoles/min/g fresh weight. Abbreviations were RDP, ribulose 1,5-diphosphate; G 3-P, glyceraldehyde 3-phosphate; PEP, phosphopyruvate.

Light-dependent Synthesis of Enzymes in Dark-grown Leaves

When maize plants were grown in total darkness and then transferred to the light the contents of chlorophyll and the Calvin Cycle enzymes ribulose 1,5-diphosphate carboxylase and glyceraldehyde 3-phosphate dehydrogenase increased (Table 4). Similar increases in activity were observed with pyruvate, P_i dikinase, phosphopyruvate carboxylase, adenylate kinase and pyrophosphatase. After 35 hr in the light the activities of these enzymes and the chlorophyll content had almost reached the values for leaves of control plants grown under normal light conditions from germination. There were no changes in the activities of malate dehydrogenase or acid phosphatase. These and other studies provided no evidence that chlorophyll synthesis was a prerequisite of enzyme formation.

DISCUSSION

Like other metabolic processes, photosynthesis is probably regulated by a range of control processes. Separate unpublished studies have shown that both pyruvate, P_i dikinase (T. J. Andrews and M. D. Hatch) and phosphopyruvate carboxylase (J. Lowe and C. R. Slack) from sugar cane or maize leaves are subject to product inhibition. Furthermore, pyruvate, P_i dikinase is inactivated in the dark and reactivated upon re-illumination of leaves, the extent of reactivation being related to light intensity.[11, 12] These changes occur with a half-time of about 10–15 min and may together with the product-inhibitor effects provide rapidly-acting means of regulation.

The present studies provide evidence for another type of regulatory process which operates in response to more prolonged changes of light intensity. Of the photosynthetic enzymes examined the most pronounced changes were observed with pyruvate, P_i dikinase and phosphopyruvate carboxylase, enzymes involved in the CO_2-fixation phase of the C_4-dicarboxylic acid pathway. Some other enzymes changed to a lesser extent but the activity of ribulose 1,5-diphosphate carboxylase was unaffected. As shown in Fig. 1 the potential for photosynthesis in plants with the C_4-dicarboxylic acid pathway is strongly light-limited below 60,000 lux, at least for plants grown in sunlight. At 8600 lux the potential rate is between $\frac{1}{5}$ and $\frac{1}{10}$ of the maximum rate of photosynthesis. It is noteworthy that the activites of pyruvate, P_i dikinase and phosphopyruvate carboxylase underwent changes of similar magnitude when plants were transferred from 130,000 lux to 8600 lux or vice versa. An interpretation of these findings is that enzyme activities adjust to levels sufficient to support photosynthetic CO_2-fixation at the maximum rate permitted by the available light energy. The changes in the photosynthetic characteristics of attached leaves, following adaptation to low light, can therefore be interpreted as a change in the balance of the potential rates of the light-dependent phase and the CO_2-fixation phase of photosynthesis. If, as the results indicate, the potential rate for the light-dependent phase remains relatively constant, then the changes in the maximum CO_2-fixing capacity and light-saturation characteristics for photosynthesis could be explained entirely by a reduction in the potential of the CO_2-fixation phase. It is of interest that the characteristics of the curve for plants adapted to low light closely resembles both quantitatively and qualitatively the light-saturation curves for Calvin Cycle plants.[7]

Our earlier studies of $^{14}CO_2$ fixation by leaves suggested that the contribution of ribulose 1,5-diphosphate carboxylase to the fixation of externally-supplied CO_2 is very small or insignificant in plants in which the C_4-dicarboxylic acid pathway is operative.[1, 2] This conclusion was supported by other studies which showed that the plants with the C_4-dicarboxylic acid pathway contain levels of this enzyme which are as low as $\frac{1}{20}$ of that in

Calvin Cycle plants, relative to maximum photosynthesis rates.[4] If ribulose 1,5-diphosphate does contribute to fixation of externally-derived CO_2 we would predict that its relative contribution would be much greater in maize or *Amaranthus* plants grown for a period at a low light intensity. Under these conditions the content of the two enzymes associated with CO_2-fixation in the C_4-dicarboxylic acid pathway was reduced many fold but the activity of ribulose 1,5-diphosphate carboxylase remained unaffected. A comparison of the labelling patterns of plants adapted to low light with those of plants grown under standard sunlight conditions provided no evidence for any increased contribution by this enzyme. The possibility that ribulose 1,5-diphosphate carboxylase does not fix externally-derived CO_2, but may fix internally-derived CO_2, in these plants was supported by recent studies on its distribution between the two types of chloroplasts present in maize and *Amaranthus*.[13] The relatively small quantity of this enzyme present in these plants was at least predominantly located in the chloroplasts of the bundle-sheath layer of cells. In contrast mesophyll chloroplasts contained most of the pyruvate, P_i dikinase and most of the chlorophyll and for maize at least it has been calculated[14] that these chloroplasts contain about 90% of the photosynthetic membrane surface of the leaf.

We have suggested[5] that adenylate kinase and pyrophosphatase may have specific roles in the C_4-dicarboxylic acid pathway by removing pyrophosphate and AMP, products of the reaction catalysed by pyruvate, P_i dikinase. Our present studies provide support for the view that these enzymes have a role in photosynthesis. The content of these enzymes was shown to be much higher in leaves with the C_4-dicarboxylic acid pathway and was more than adequate to account for the rates of photosynthesis of attached leaves. Although these enzymes would almost certainly function in other phases of metabolism within the leaf it would appear that a major part of the total leaf complement could operate in the manner proposed.

Studies of another type provided further evidence that adenylate kinase and pyrophosphatase and also pyruvate, P_i dikinase and phosphopyruvate carboxylase are photosynthetic in character. The activities of these enzymes were low in leaves of dark-grown plants and increased approximately 10-fold following exposure to light. The increases observed exceeded those for ribulose 1,5-diphosphate carboxylase and glyceraldehyde 3-phosphate dehydrogenase. These results support the view that the former enzymes are both physically and functionally associated with the photosynthetic apparatus of the leaves. Similar observations have been made with other photosynthetic enzymes and recent studies with algae[15] and detached leaves[16,17] suggest that these increases may be due to synthesis of enzyme within the developing chloroplast. Of course, our early studies[4,13] have shown that at least a large part of phosphopyruvate carboxylase, pyruvate-P_i dikinase and adenylate kinase of leaves is associated with chloroplasts isolated in non-aqueous media.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* var. DS606A) and *Amaranthus palmeri* were grown in a vermiculite-perlite mixture watered with nutrient solution. Plants were grown at 28° with a 12 hr light

[13] C. R. SLACK, *Phytochem.*, in press (1969).
[14] A. M. SILAEVA, *Fiziologiya Rastenii* **13**, 623 (1966).
[15] R. M. SMILLIE, D. GRAHAM, M. R. DWYER, A. GRIEVE and N. F. TOBIN, *Biochem. Biophys. Res. Commun.* **28**, 604 (1967).
[16] S. CHEN, D. MCMAHON and L. BOGORAD, *Plant Physiol.* **42**, 1 (1967).
[17] C. J. KELLER and R. C. HUFFAKER, *Plant Physiol.* **42**, 1277 (1967).

period of either daylight (approx. 130,000 lux) or artificial light (8600 lux obtained with 400 W Philips H.P.L. mercury-vapour lamps supplemented with tungsten lamps). To facilitate the establishment of plants subsequently grown at low light intensity, germination and growth was allowed to proceed for 2–3 weeks in sunlight. Light measurements were made with a calibrated selenium cell as previously described.[1]

Chemicals

The sources of radiochemicals, biochemicals and reagent enzymes were as previously described.[4, 5]

Light Intensity Treatments

The general conditions for growth are described above. Sunlight was used for the high light-intensity treatments, being the only practical source. The 6 days over which the critical part of the experiments described in Tables 2 and 3 and Fig. 1 were conducted was essentially cloudless. Under these conditions the light intensity would be 130,000 lux or more for at least 9 hr each day.

Extraction and Assay of Enzymes

Leaves were removed from plants and immediately sub-sampled, weighed into 1·5 g lots, and ground for 1 min in a chilled mortar with 4 volumes (w/v) of 0·1 M-tris-HCl, pH 8·3, containing 10 mM-$MgCl_2$, 10 mM-dithiothreitol and 1 mM-EDTA, together with 1 g of sand. Extracts were filtered through mira-cloth and samples were treated on a small column of Sephadex G-25, previously equilibrated with 0·05 M tris-HCl, pH 8·3, containing 5 mM dithiothreitol and 10 mM $MgCl_2$, to remove small mol. wt. compounds from the original extract. Samples of this extract were used for the assay of enzymes. The chlorophyll content of leaf extracts was determined[18] prior to the treatment on Sephadex G-25.

Adenylate kinase was assayed by measuring the change of absorbance at 340 mμ in reaction mixture containing 5 mM phosphopyruvate, 0·5 mM ATP, 0·5 mM AMP, 2 mM $MgCl_2$, 50 mM KCl, 20 mM sodium *N*-2-hydroxethylpiperazine-N^1-2-ethanesulfonic acid buffer, pH 7·7, 0·2 mM $NADH_2$ and excess of the enzymes pyruvate kinase and lactate dehydrogenase. The reaction was started by adding leaf enzyme after changes in the absorbance due to traces of pyruvate or ADP in the reagents had ceased.

Pyrophosphatase was measured at its pH optimum of 8·9 in reaction mixtures containing 2 mM sodium pyrophosphate, 8 mM $MgCl_2$ and 50 mM tris-HCl buffer pH 8·9. Reactions were stopped by the addition of trichoroacetic acid to give a final concentration of 2% (w/v). Orthophosphate formed was measured as described by Lowry.[19] Other enzymes were assayed as previously described[4, 5] except that where applicable 2-mercaptoethanol was replaced by the same concentration of dithiothreitol. Spectrophotometric assays were conducted at 24° and all other assays at 30°. The procedure for converting inactive pyruvate, P_i dikinase to its active form in leaf extracts is described elsewhere.[12]

Photosynthesis of Attached Leaves

Photosynthesis rates were determined on single leaves attached to the plant by measuring the difference between the input and output concentrations of CO_2 in air flowing through

[18] D. I. Arnon, *Plant Physiol.* **24**, 1 (1949).
[19] O. H. Lowry, in *Methods in Enzymology* (edited by S. P. Colowick and N. O. Kaplan) Vol. 4, p. 373, Academic Press, New York (1957).

the leaf chamber. The apparatus was essentially the same as described by Bierhuizen and Slayter.[20] The light source was a 1500 W quartz-iodine lamp, and light intensity was regulated by varying the voltage supplied to the lamp.

$^{14}CO_2$-*Fixation by Detached Leaves*

$^{14}CO_2$ was provided to leaves photosynthesizing at a steady rate and leaves were killed at the times indicated by transfer to boiling 80% (v/v) ethanol. The details of this procedure and of the methods for analysis of the distribution of radioactivity in individual compounds were as previously described.[1,2]

[20] J. F. BIERHUIZEN and R. O. SLAYTER, *Australian J. Biol. Sci.* **17**, 348 (1964).