Plant Physiol. (1984) 75, 397–409
0032-0889/84/75/0397/13/$01.00/0

Photosynthetic Carbon Metabolism in Leaves and Isolated Chloroplasts from Spinach Plants Grown under Short and Intermediate Photosynthetic Periods

Received for publication September 27, 1983 and in revised form January 31, 1984

J. MICHAEL ROBINSON*
Light and Plant Growth Laboratory, Plant Physiology Institute, United States Department of Agriculture, Science and Education Administration, Agricultural Research, Beltsville Agricultural Research Center-West, Beltsville, Maryland 20705

ABSTRACT

Responses of foliar and isolated intact chloroplast photosynthetic carbon metabolism observed in spinach (*Spinacia oleracea* cv Wisconsin Bloomsdale) plants exposed to a shortened photosynthetic period (7-hour light/17-hour dark cycle), were used as probes to examine *in vivo* metabolic factors that exerted rate determination on photosynthesis (PS) and on starch synthesis. Compared with control plants propagated continuously on a 12-hour light/12-hour dark cycle, 14 to 15 days were required, subsequent to a shift from 12 to 7 hours daylength, for 7-hour plants to begin to grow at rates comparable to those of 12-hour daylength plants. Because of shorter daily durations of PS, daily demand for photosynthate by growth processes appeared to be greater in the 7-hour than in the 12-hour plants. The result was that 7-hour plants established a 1.5- to 2.0-fold higher total PS rate than 12-hour plants.

Intact chloroplasts isolated from the leaves of 7-hour plants (7-h PLD) displayed 1.5- to 2.0-fold higher PS rates than plastids isolated from 12-hour plants (12-h PLD). Plastid lamellae prepared from 7- and 12-h PLD isolates displayed equivalent rates of ferredoxin-dependent ATP and NADPH photoformation indicating that electron transport processes were not factors in the establishment of higher 7-h PLD PS rates. Analyses, both in leaves as well as intact PLD isolates, of dark to light transitional increases in Calvin cycle intermediates, *e.g.*, ribulose-1,5-bisphosphate (RuBP) and 3-phosphoglycerate (3-PGA), as well as estimations of activities of RuBP carboxylase and fructose-1,6-bisphosphate phosphatase, indicated that 7-hour plant leaves displayed higher PS rates (than 12-hour plants), because there was a higher magnitude of activity of the Calvin cycle.

Although both the foliar level of starch and sucrose, as well as starch synthesis rate, often was higher in 7-hour compared with 12-hour plant foliage, the higher 7-hour plant total PS rates indicated that maximal sucrose and starch levels did not mediate any 'feedback' inhibition of PS. The higher 7-hour plant foliar and PLD PS rates resulted in higher glucose-1-P levels as well as a higher ratio of 3-PGA:Pi, both factors of which would enhance the activity of chloroplast ADP-glucose pyrophosphorylase, and which were attributed to be causal to the higher starch synthesis rates observed in 7-hour plant foliage and PLD isolates.

Leaf photosynthetic rate may be regulated by the requirement for photosynthate, *i.e.* the 'demand' for photoassimilate, exerted by the growth and development of sink organs in the higher plant (8, 11). Silvius *et al.* (28) observed that when soybean trifoliolate leaves reached maximum area accretion, PS[1] decreased, and sucrose accumulation approached maximum levels. Thorne and Koller (31) demonstrated that increasing photosynthate demand on one soybean trifoliolate, caused by shading all other leaves, resulted not only in an increased net PS rate which correlated with an increased RuBP carboxylase activity, but also a decrease in levels of starch and soluble carbohydrates. Conversely, Mondal *et al.* (16) found that removal of developing soybean organs, including floral buds and bean pods, resulted in a diminution of PS rate, a decrease in RuBP carboxylase activity, and an accumulation of starch and soluble carbohydrates in the leaves of 'desinked' plants. There have been suggestions that enhanced accumulation of reserve foliar carbohydrates exerted a 'feedback' inhibition of photosynthetic carbon metabolism enzymes such as RuBP carboxylase in source leaves (8, 11, 16). Inhibition ostensibly could be mediated by metabolites and/or hormones, but evidence for such control of PS at the intact leaf level is lacking (8, 11). Additionally, RuBP carboxylase is not the only rate-determining enzyme in the Calvin cycle, and other enzymes in that pathway, *e.g.* FBP phosphatase, could serve to limit the regeneration of RuBP thereby influencing the total PS rate (32). In the intact leaf, control of PS could be at many sites in the pentose phosphate reductive cycle (11, 32).

There is evidence that an enhanced accumulation of starch and soluble nonstructural carbohydrate does not trigger a diminution of PS (8, 11). Recent work in several laboratories (3–5, 12, 21, 22) demonstrated that C-3 and C-4 plant species, grown on a 7-h light/17-h dark regime accumulated a higher level of starch and soluble nonstructural carbohydrates when compared to leaves of plants maintained on a 12-h light/12-h dark cycle, while foliar PS rate was similar in both acclimates. In the case of soybean (3), it was found that not only was there an enhanced accumulation of starch and reserve sugars in the 7-h plant leaves, but there was an apparent increase in PS when the rate was expressed on a dry weight basis (3). In that study (3), it appeared that 7-h daylength plant leaves remained strong photosynthate sinks and simultaneously exhibited an accumulation of nonstructural carbohydrates. Additionally, Servaites and Ogren (25)

[1] Abbreviations: PS, photosynthesis; SLW, specific leaf weight; PR, photorespiration; DR, dark respiration; CO_2, CO_2 (gas) atmospheric or dissolved in solution; HCO_3, bicarbonate; 'CO_2', CO_2 + HCO_3; 3-PGA, 3-phosphoglycerate; GAP, glyceraldehyde-3-P; DHAP, dihydroxyacetone-P; triose-P, GAP + DHAP; GlP, glucose-1-P; G6P, glucose-6-P; F6P, fructose-6-P; FBP, fructose-1,6-bisP; RuBP, ribulose-1,5-bisP; ADP-glucose, adenosine diphosphate glucose; 7-h PLD/12-h PLD, intact plastids isolated from the leaves of plants adapted, respectively, to a 7-h light/17-h dark cycle, or to a 12-h light/12-h dark cycle.

found that isolated soybean leaf mesophyll cells displayed highest net PS rates when the cells were prepared from plants propagated on a shortened daylength (8 h). This observation (25) suggested that the higher foliar PS rate response to the shortened daylength (3) was caused by a higher magnitude of carbon metabolism in the chloroplast.

Since the photosynthetic daylength influenced the magnitude of net PS as well as the magnitude and rate of accumulation of total nonstructural carbohydrate, I reasoned that exposure of plants to short and intermediate daylength could serve as a tool to facilitate the examination of metabolic factors that potentially control PS, both at the foliar and at the chloroplast level. This study, then, used the shortened and intermediate daylength responses elicited by spinach plants to examine metabolic factors that may potentially control PS rate, *e.g.* photosynthate requirements (demands) exerted by plant growth and development, foliar starch and sucrose level, photosynthetic ATP and NADPH production, and/or magnitude of pentose phosphate reductive cycle metabolism. Compared with control spinach plants propagated on a 12-h light/12-h dark cycle, it required 15 d for the growth rate to increase in 7-h light/17-h dark-exposed plants. As growth began to improve in the 7-h daylength plants, responses of photosynthetic metabolism in both the 7- and 12-h plants were monitored. As maturity was attained in the 12-h plants, PS and DR diminished; simultaneously, 7-h plants matured more slowly and PS and DR rates remained maximal. Regardless of the magnitude of sucrose and starch level in the 7-h plants, the PS and DR rate remained maximal, indicating that (a) it was the requirements by growth for photoassimilate that resulted in sustained high PS rates in the 7-h plants, and (b) starch and sucrose level had no effect on PS rate.

A comparison of PS and carbon metabolism in preparations of isolated intact chloroplasts from 7- and 12-h plant foliage indicated that, in the intact leaf chloroplast, it was the magnitude of activity of several enzymes of the Calvin cycle, *e.g.* RuBP carboxylase and FBP phosphatase, and not the magnitude of production of photosynthetic electron transport products (ATP, NADPH) which determined the magnitude of foliar total PS. Additionally, in 7-h plant leaves and isolated chloroplasts, a combination of factors appeared to favor an increased rate of starch synthesis. These included (a) the higher rate of PS which resulted in there being both a higher level of GIP and a higher ratio of 3-PGA/Pi, metabolic conditions which favored a high activity of ADP-glucose pyrophosphorylase, an enzyme which is rate limiting to the chloroplast starch synthesis pathway (9, 18); and (b) in some cases, the absolute magnitude of activity of ADP-glucose pyrophosphorylase from spinach chloroplasts was higher in 7-h compared with 12-h plant plastid isolates.

Preliminary results from these studies have been presented (21, 22).

MATERIALS AND METHODS

Plant Material and Daylength Manipulation. *Spinacia oleracea* cv Wisconsin Bloomsdale plants were propagated from emergence in Environmental Growth Chambers[2] with either a 7-h light/17-h dark or a 12-h dark period (24-h cycle). In some experiments, plants were propagated on a 12-h light/12-h dark regime for 10 d postemergence. At that time, one-half of the plant lot was switched to a 7-h light/17-h dark regime for the remainder of the growth period.

Each growth chamber was fitted with 24, 2.44-m, 210-w Sylvania VHO or Westinghouse SHO fluorescent bulbs mixed with 10, 60-w incandescent bulbs. Incident light intensity was 500 $\mu E/m^2 \cdot s$ and growth temperature was 25°C day/night. RH in all chambers was 65 ± 2%. Plants were propagated in vermiculite in 15-cm plastic pots, and these were surface irrigated daily with a nutrient solution (developed by Dr. F. W. Snyder in this laboratory). The nutrient solution contained (mM): $Ca(NO_3)_2 \cdot 4H_2O$, 4.0; KNO_3, 4.0; $NH_4H_2PO_4$, 1.0; NH_4Cl, 1.5; KCl, 2.5; K_2SO_4, 1.0; and $MgSO_4 \cdot 7H_2O$, 3.5. Micronutrient concentrations (μM) were: H_3BO_3, 20.6; $CuSO_4 \cdot 5H_2O$, 0.16; $MnSO_4 \cdot 2H_2O$, 4.5; $(NH_4)_6Mo_7O_{24}$, 0.07; $ZnSO_4 \cdot 7H_2O$, 0.34; and Fe Chelate, 107.4 as Sequestrene 330 (CIBY-GEIGY Corp.).

The 12-h or shorter daylength permitted continuous vegetative growth, because spinach plants require at least 12.5 to 13.0 h daylength for initiation of flowering.

Leaf Photosynthesis, Photorespiration, and Dark Respiration. Net PS was determined for single leaves employing a modification of the 'air seal' technique developed by Wolf *et al.* (33). Attached, whole spinach leaves were placed in acrylic measuring chambers (20 × 15 × 2.5 cm) for gas flow through to an IR CO_2 analyzer (Beckman 865). Airflow in the system was monitored using a mass flow meter (Teledyne Hastings). Measurements of gas exchange were conducted within the growth chambers with 330 $\mu l/l$ CO_2, 500 $\mu mol/m^2 \cdot s$ of incident light (400–700 nm) upon the measuring cuvette, and 25°C within the cuvette.

Measurements of PR and DR (on the same leaves measured for PS) were made by monitoring CO_2 release into CO_2-free air. The measuring chamber was flushed with CO_2 free air, the evolution of CO_2 in the light was monitored, the growth chamber lights were extinguished, and the postillumination CO_2 release (burst) was measured, followed by continuous measurement of DR. Actual PR values were taken as the values for the PR measured plus the values for the postillumination CO_2 burst measured minus the DR measured. Total PS in the light was computed for each measured leaf as the sum of net PS plus PR plus DR; dark or mitochondrial respiration has been shown to be fully functional in the light (1).

Leaf Sampling. Following PS, PR, and DR measurement, leaves were removed, immediately weighed, and leaf area was estimated employing a LI-COR model LI-3000 area meter. The leaves were placed in small paper envelopes, submerged in liquid N_2 for 1 to 2 h, and lyophilized for 96 h. The dried leaves were weighed (for SLW determination) and Chl was extracted and quantitated, this facilitated the expression of PS, PR, and DR rate as well as intermediate levels on either an area, dry weight, or Chl basis.

In experiments where leaf and stem tissues were harvested for determination of photosynthetic intermediates, sucrose and starch, surgical removal (scissors) of tissues was carried out directly within the growth chambers at specific time points in the dark or light. Excised tissues were submerged directly into liquid N_2 (contained in liter pyrex beakers), the frozen tissues were agitated with glass rods until the tissue particles were powdered, the powdered tissue was rapidly transferred to envelopes held in the liquid N_2, and the envelopes were placed in the freeze-dryer for lyophilization as previously described. Lyophilized tissues were stored desiccated at −20°C, and large veins were removed from the lyophilized samples prior to tissue extractions.

Leaf Chl. Chl was extracted from 10 to 15 mg freeze-dried leaf tissue with 10 ml 80% acetone, and Chl was quantitated as described previously (23).

Phosphorylated Intermediates in Leaves. Extraction and measurement of RuBP, 3-PGA, GAP + DHAP, FBP, F6P, G6P, and G1P from 75 to 100 mg freeze-dried leaf tissue was accomplished using the methods of Latzko and Gibbs. (15).

Foliar Sucrose and Starch. Sucrose and starch were extracted from 18 to 20 mg freeze-dried leaf, stem, and root tissue em-

[2] Mention of a trademark, proprietary product, or vendor does not constitute guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may be also suitable.

ploying the methods developed by Dickson (6). Released glucose from starch (amyloglucosidase) or from sucrose (invertase) fractions were converted enzymically to G6P, and quantitated employing the methods of Latzko and Gibbs (15).

Foliar and Isolated Plastid Protein. Buffer-soluble protein was extracted from 15 mg of freeze-dried leaf tissue in 2 to 3 ml of 50 mM Hepes (pH 7.0), the homogenate was centrifuged at 2000*g* for 10 min, and soluble protein was quantitated in 0.1-ml aliquots of the resulting supernatant using the Bio-Rad Protein Assay Reagent (Bio-Rad Technical Manual No. 82-0275).

Buffer-soluble protein was estimated in the stromal fractions prepared from intact plastid isolates. Isolates of chloroplasts containing intact plastids were prepared employing the methods cited below (20). After centrifugation, the resulting pellet from the sorbitol homogenizing medium (20) was resuspended in 2 to 4 ml 50 mM Hepes (pH 7.0) for osmotic disruption, and the suspension was centrifuged at 4000*g* for 20 min. Protein in solution was estimated in 0.1-ml aliquots of the resulting supernatant, and Chl was estimated on the resulting pellet.

Pi. Fifteen mg of freeze-dried leaf tissue was extracted 3 × with 3 ml of 5% (w/v) TCA, the consecutive extracts were centrifuged at 1000*g* for 10 min, the combined supernatants were diluted to 25 ml with glass-distilled H_2O, and Pi was quantitated in 2 ml of extract employing the Fiske-Subbarow reagent (7).

PS in Isolated Chloroplasts. Intact leaf chloroplasts were isolated from the leaves of spinach plants adapted to the 7- or 12-h daylength employing the methods of Robinson *et al.* (20). Subsequent to plastid isolation, plastid intactness was assayed using either the ferricyanide or the Fd-NADP method (20).

Prior to isolation of chloroplasts, plants were transferred from the growth chamber to the laboratory. Mature leaves were excised, washed, and blotted and intact plastids were prepared. The process required approximately 30 to 60 min from removal of plants from the growth chambers to addition of intact plastids to reaction mixtures. Experiments measuring photosynthetic parameters in plastid preparations from leaves of plants adapted to 7- and 12-h light regimes were conducted on the same day. This necessitated isolating plastids and conducting measurements of plastid photosynthesis for each daylength acclimate at a different period in the day. Regardless of the time of day of plastid isolation, those trends of PS rate displayed in Table III and Figures 1 and 4 were observed routinely, *i.e.* higher PS rate in 7-h compared with 12-h PLD isolates.

Chloroplasts isolated from both 7- and 12-h adapted plants contained the same ratio of stromal protein to intact plastid Chl. For example, in one preparation a 0.1-ml aliquot of 7-h PLD contained 21.7 μg Chl (associated with the intact plastids) and 443.3 μg soluble protein; the ratio of protein/Chl in the 7-h PLD was 20.5. In the 12-h PLD, the stromal protein/Chl = 444.0/21.9 or 20.3. Although the amount of Chl and protein per preparation was variable from experiment to experiment, the 7- and 12-h PLD stromal protein/Chl ratios within an experiment were similar in 16 separate studies.

Fd-dependent O_2, NADPH, and ATP photoformation was estimated in preparations of plastid lamellae prepared directly from the 7- and 12-h PLD isolates. Chloroplast lamellae isolation techniques as well as methods of simultaneous monitoring and quantitation of O_2, ATP, and NADPH photoformation were described previously (23). PS parameters for both intact plastid isolates and lamellae isolates were estimated in the same experimental period (see Table III).

Light-dependent $^{14}CO_2$ assimilation into acid-stable products in PLD isolates was monitored using the methods described previously (19, 20), except that reaction mixtures were not aerated with compressed air, and $^{14}CO_2$ fixation was quantitated using scintillation spectrophotometry. Reaction mixture composition is described in the Figure 4 legend. All rates of $^{14}CO_2$ photoassimilation are corrected to the basis of 100% intact plastids in each reaction mixture.

Products of photosynthesis were separated employing one-dimensional chromatography described previously (19), and in some cases [^{14}C]glycolate was separated employing Dowex-acetate ion-exchange chromatography (19, 20). Polyglucan or starch, synthesized during $^{14}CO_2$ assimilation, was quantitated employing either one-dimensional paper chromatography (19) or the method outlined by Steup *et al.* (29) except that *n*-butanol (rather than ethanol) was used to wash the filters (Millipore HAWP 02500, 0.45-μm pore size) in order to remove all products except [^{14}C]amylose and amylopectin.

Chloroplast Enzymes. Enzyme activities were measured in isolates of intact plastids after osmotic disruption in the measuring cuvette. In all cases, assays were made concomitant with other measurements of intact plastid photosynthesis, and all activities were corrected to a mg Chl and 100% intact plastid basis. Assays were carried out at 25°C in 50 mM Hepes-NaOH (pH 8.1). Mixtures were air equilibrated prior to assays.

(a) RuBP Carboxylase (Method One). RuBP-dependent, $^{14}CO_2$ fixation in plastid isolates was assayed using the method of Bahr and Jensen (2).

(b) RuBP Carboxylase (Method Two). Activity was measured employing the method of Lilley and Walker (16) with the CO_2 activation step described by Bahr and Jensen (2). After the initial 2- to 3-min lag period, activity was linear for as much as 15 min.

(c) Fructose-1,6-bisP Phosphatase. Activity was monitored employing the procedures of Kelly *et al.* (13). Reactions displayed an initial 5- to 7-min lag period after which linearity was attained for the remainder of the assay (additional 13 min).

(d–g) Phosphoglycerate Phosphokinase, NADPH Triose Phosphate Dehydrogenase, Transketolase, and Phosphoribulokinase. These enzyme activities were measured according to Latzko and Gibbs (14).

(h) ADP-Glucose Pyrophosphorylase. Activity of this enzyme was assayed employing the methods of Ghosh and Preiss (9) and Shen and Preiss (26). Reaction mixtures contained, in 1.0 ml: 50 mM Tricine, pH 8.1; 5 mM $MgCl_2$; 1 mM DTT, 1 mM 3-PGA, 0.25 mM K_2HPO_4-K_2PO_4; 1 mM ATP; 1 mM [U-^{14}C]glucose-1-P (0.04 μCi), and intact plastids equivalent to 30 to 100 μg Chl. Reactions were initiated by ATP (there was 1 to 5% activity in the absence of ATP). Samples (250 μl) were removed at 0, 3, 6, and 12 min, brought to 100°C and held at that temperature for 10 min. ADP-glucose was purified and quantitated according to previously described methods (26).

RESULTS

Influence of Shortened Photosynthetic Duration upon Spinach Plant Growth. *Plant Growth, Shoot/Root Ratio, SLW, and Chl.* Plant growth was examined in relation to the influence that the daily duration of PS exerted upon dry weight accumulation and leaf area accretion. Spinach plants were propagated through 10 d postemergence on a 12-h daylength. Beginning on the 11th d, one-half of the plants were transferred to a 7-h light/17-d dark cycle, and the plants were sampled at 25, 32, and 39 d postemergence, *i.e.* 15, 22, and 29 d exposure to a 7-h daylength with control plants growing continuously on a 12-h daylength (Table I).

After the first 15 d of exposure of the test plants to 7-h daylengths, total dry weight accretion in the 7-h compared with the 12-h plants was 80% reduced (Table I). In contrast, during the 7-d period between 15 and 22 d adaptation, 7-h plants displayed increased growth rate when compared with the 12-h controls. Average total dry matter accumulation in that period was for 7- and 12-h plants, respectively, 358.6 and 475.7 mg/d plant which represented only a 26% slower growth in the 7-h plants. In that period, there was, for the 7- and 12-h plants,

Table I. *Characteristics of Spinach Plant Growth on a 7-h Light/17-h Dark Compared with a 12-h Light/12-h Dark Cycle*

S. oleracea cv Wisconsin Bloomsdale were propagated in the growth chamber from emergence on a 12-h light/12-h dark regime for 10 d at which time one-half of the plants were shifted to a second growth chamber with a 7-h light/17-h dark regime for the remainder of the test period (see "Materials and Methods"). Plants were sampled simultaneously in both regimes at 25, 32, and 39 d old (15, 22, and 29 d on 7-h light adaptation time). Growth measurements were conducted according to procedures described in "Materials and Methods." In this study, the term stems pertains to petioles plus crown tissue.

Treatment	Total Leaf Area	Total Dry Wt/Plant				Ratio: Leaves + Stems/ Roots
		Leaves	Stems	Roots	Total	
	cm^2/plant	mg				
25 d old						
10 d, 12 h; 15 d, 7 h	340.0 ± 45.5	1425 ± 215	195 ± 15	190 ± 20	1810 ± 250	8.5 ± 0.5
25 d, 12 h	750.0 ± 23.8	4935 ± 85	575 ± 75	1010 ± 210	6520 ± 200	5.7 ± 1.2
32 d old						
10 d, 12 h; 22 d, 7 h	611.9 ± 6.8	3015 ± 165	525 ± 15	780 ± 30	4320 ± 180	4.5 ± 0.0
32 d, 12 h	874.4 ± 28.6	6540 ± 480	1115 ± 115	2195 ± 285	9850 ± 880	3.5 ± 0.2
39 d old						
10 d, 12 h; 29 d, 7 h	938.6 ± 26.2	4900 ± 190	970 ± 45	1260 ± 25	7130 ± 215	4.7 ± 0.1
39 d, 12 h	2530.4 ± 294.6	14850 ± 283	1720 ± 95	4180 ± 248	20750 ± 540	4.0 ± 0.1

respectively, a 2.4- and a 1.5-fold increase in total dry matter. However, in the ensuing period between 22 and 29 d acclimation there was, in the 7-h plants, only a 1.7-fold increase in total dry weight, but in the 12-h plants there was a 2.1-fold increase. Certainly 7-h plant growth in this later period was more successful than in the first 15 d (Table I).

Dry matter was preferentially distributed to the stems and leaves of the 7-h plants, a characteristic of shortened daylength adaptation in higher plants (3, 10). This was reflected in the higher shoot/root ratio in the 7-h compared with the 12-h plants in all sampling periods, and this indicated preferred growth in the shoots relative to the roots in the 7-h plants (Table I). However, there was the indication that root growth relative to shoot growth increased as 7-h daylength exposure continued, *e.g.* at 15 d acclimation the shoot/root ratio was 1.5 times greater in the 7-h than in the 12-h plants. After 29 d exposure, that factor was different by approximately 1.2 times (Table I), and this also reflected the improvement over time in the development of the 7-h plants.

In most mature 7-h plant leaves, SLW was 40% lower than in the 12-h plant foliage (Table II) (3, 20). This indicated that, per dm^2, leaves of 7-h plants were thinner than those of 12-h plants.

In all cases tested, the amount of Chl/g dry weight (freeze-dried) was the same in mature leaves of 7-h relative to 12-h plants, *e.g.* 12 to 14 mg/g dry weight (Table II). Since there was a lower SLW for 7-h plants, there was 32 to 45% less Chl/dm^2 relative to the 12-h plant leaves (Table II). Assuming there was the same amount of Chl/chloroplast in both 7- and 12-h foliage, then the 7-h leaf foliage had 32 to 45% less plastid/dm^2 than the 12-h plant leaves.

Influence of Shortened Photosynthetic Daylength upon Foliar Photosynthesis, Respiration, and Carbon Metabolism. The metabolic changes of foliar photosynthetic metabolism elicited in response to growth on a 7- or 12-h daylength was used as a probe to search for metabolic controls upon *in vivo* PS.

Expression of Photosynthetic Rate on a Chl Basis. In order to examine responses of leaf photosynthetic rate to 7- and 12-h daylength exposure, it was necessary to measure net and total PS as well as PR and DR rate on both a leaf area (dm^2) as well as a Chl basis. Unit leaf area basis reflected PS of the population of plastids functioning within (under) a unit of leaf surface. In contrast, expression of photosynthetic rate on a Chl basis better reflected the activity of chloroplasts as individuals in the population, and facilitated the comparison of leaf PS with isolated intact chloroplast PS.

Since Chl was found to be identical, per mg dry weight of leaf tissue, in both 7- and 12-h daylength exposed plants (Table II), it was valid to express PS, PR, and DR rate on a Chl basis. Additionally, it was found that the magnitude of NADPH and ATP photoformation in isolates of plastid lamellae prepared from 7- and 12-h plants were identical (Table III); this also indicated that it was valid to compare 7- and 12-h foliar PS, PR, and DR rates on a Chl basis (Table II).

Influence of Shortened Daylength upon Leaf Photosynthetic Rates. Spinach plant growth was severely restricted during the first 15 d of adaption on the 7-h light regime (Table I). At least one reason for that growth reduction was that during the first 8 to 12 d of adaptation to 7-h light/17-h dark cycles, total PS was much lower in rate when compared to the 12-h plants. For example, total PS in 8- to 12-d adapted 7-h plant leaves exhibited rates of 54 to 70 μmol CO_2 assimilated/h·mg Chl, while rates in 12-h plant counterparts were 82 to 99 μmol/h·mg Chl (data not shown).

Beginning at the 15th d of acclimation and concomitant with improvement in growth rate in the 7-h plants, there was an increase in the photosynthetic rate, and this was best observed on a Chl basis (Table II). In Table II, it was shown that because SLW was much lower in the 7-h plants, the rate of photosynthesis on an area basis (dm^2) appeared to be much lower in the 15-d, 7-h acclimates. Seven-h plant PS was, in fact, approximately the same or slightly higher than that of 12-h plant foliage.

During the next subsequent 7-d intervals, total PS displayed rates of approximately 100 to 115 μmol CO_2 assimilated/h·mg Chl in the 7-h leaves, but the rates of PS in the 12-h plants declined to values of 48 to 59. Even though SLW was lower in the 7-h leaves, rates of PS/dm^2 were higher, due to the decline in the 12-h plant PS rates (Table II). Improved growth rates in the 7-h plants during the 15- to 29-d acclimation were the results of the plants' increased plastid PS (Tables I and II).

In other studies, it was observed that even after 50 to 74 d of acclimation to 7-h daylength, net and total PS could remain higher in the leaves of shorter daylength plants. When net PS was measured on spinach plants acclimated for 55 d to 7-h light/17-h dark cycles, the average net PS exceeded that of 12-h plants by a factor of 1.3- to 1.4-fold. With conditions set at 25°C, 330 μl/l CO_2 and 500 $\mu E/m^2 \cdot s$, intact 7-h plants displayed hourly CO_2 assimilation rates on dm^2, g dry weight, or mg Chl basis of, respectively, 348.1, 728.0, 57.3 μmol CO_2/h·unit. In the 12-h controls, the rates, on an area, dry weight, and Chl basis were, respectively, 243.3, 444.5, and 31.9 (data not shown).

Table II. *Photosynthesis, Photo-, and Dark Respiration Rates for Single Leaves of Plants Described in Table I*

PS, PR, and DR rates are expressed on both a leaf dm^2 as well as a Chl basis for comparison. All measurements were made during the period from 0900 to 1300 hours. The rates are averages of a total of two or three leaves on each of two randomly selected plants. For 25-, 32-, and 39-d-old 7- and 12-h plants, the average areas of leaves measured for PS, PR, and DR were, respectively, 46.4 ± 7.8 and 66.5 ± 3.3 cm^2; 82.8 ± 3.6 and 99.6 ± 19.7 cm^2; 101.5 ± 7.7 and 174.6 ± 11.8 cm^2. The rate of water loss was, respectively, 3.7 ± 0.8 and 4.3 ± 0.7 g $H_2O/dm^2 \cdot h$; 3.7 ± 1.6 and 4.8 ± 1.1 $g/dm^2 \cdot h$; and 5.8 ± 1.0 and 2.5 ± 0.8 $g/dm^2 \cdot h$. Data entries accompanied by 'a' (7 h) and 'a' (12 h) are not significantly different, but entries accompanied by 'a' (7 h) and 'b' (12 h) were significantly different at the 95% level (*t* test).

Treatment	Specific Leaf Weight	Chl		Net Photosynthesis	Photorespiration	'Dark' Respiration	Total Photosynthesis
	mg dry wt/dm²	*μg/mg dry wt*	*mg/dm²*	*μmol CO_2 consumed or released/h·dm²*			
25 d old (15 d adapted)							
7 h	453.6 ± 28.0a	12.7 ± 1.2a	5.8 ± 0.7a	488.6 ± 124.8a	66.2 ± 18.0a	50.0 ± 21.7a	605.2 ± 147.2a
12 h	753.8 ± 39.5b	13.9 ± 0.5a	10.5 ± 0.7b	783.9 ± 86.1b	61.1 ± 9.2a	95.9 ± 14.3b	940.8 ± 90.2b
				μmol CO_2 consumed or released/h·mg Chl			
7 h				93.9 ± 22.0a	12.0 ± 4.6a	8.9 ± 4.0a	114.7 ± 22.7a
12 h				82.4 ± 13.0a	5.7 ± 0.3b	9.1 ± 2.1a	97.2 ± 12.6a
32 d old (22 d adapted)							
					μmol $CO_2/h \cdot dm^2$		
7 h	506.3 ± 13.8a	13.8 ± 0.4a	7.0 ± 0.5a	549.6 ± 86.4a	64.4 ± 19.8a	64.4 ± 29.4a	679.3 ± 126.4a
12 h	750.3 ± 69.1b	13.6 ± 1.5a	10.2 ± 1.4b	451.4 ± 36.1b	42.6 ± 7.7a	66.6 ± 16.3a	560.6 ± 54.4b
					μmol $CO_2/h \cdot mg$ Chl		
7 h				88.4 ± 18.0a	9.1 ± 2.2a	9.3 ± 4.1a	106.8 ± 22.7a
12 h				48.3 ± 5.4b	4.0 ± 0.8b	6.2 ± 1.2b	58.6 ± 4.6b
39 d old (29 d adapted)							
					μmol $CO_2/h \cdot dm^2$		
7 h	515.5 ± 58.7a	14.2 ± 0.2a	7.3 ± 0.8a	605.5 ± 135.3a	55.1 ± 14.8a	78.0 ± 26.7a	738.7 ± 157.4a
12 h	650.8 ± 44.3b	14.7 ± 0.4a	10.2 ± 1.7b	390.4 ± 234.6b	42.3 ± 31.2a	50.0 ± 37.8a	483.7 ± 303.0b
					μmol $CO_2/h \cdot mg$ Chl		
7 h				83.1 ± 20.6a	7.7 ± 2.7a	10.5 ± 2.8a	101.3 ± 23.7a
12 h				38.6 ± 22.0b	4.1 ± 3.0b	5.0 ± 3.9b	47.7 ± 30.9b

Table III. *Influence of Shortened Daylength Adaptation upon Isolated, Intact Plastid CO_2-Dependent O_2 Photoevolution and upon Electron Transport Parameters in Plastid Lamellae Isolated from the Intact Plastids*

Spinach plants were propagated in the greenhouse for 14 d postemergence, at which time one-half of the plants were transferred to the growth chambers for the 7- or 12-h illumination adaptation. The plants were held for adaptation an additional 32 d (46 d old) at which point this study was conducted. In examination of intact chloroplast photosynthesis, the reaction mixture composition was identical to that described in Figure 4 except that each mixture contained, in 1.09 ml, 1 mM DTT, 1 mM Na-isoascorbate, 500 units of catalase, and 5 mM '$^{12}CO_2$'. Chl and plastid integrity for 7-h PLD was, respectively, 23.2 μg and 41.5% intact; for the 12-h PLD, these values, were respectively, 25.4 μg and 63.5%. Rates of CO_2-dependent, O_2 photoevolution, measured in the 1- to 5-min interval, were the average of two or three repetitions and rates are expressed on the basis of 100% intact chloroplasts. Plastid lamellae were prepared from the intact plastid isolates, and O_2 photoevolution as well as ATP and NADPH photoformation were monitored simultaneously in 1.14 ml containing 50 mM Tricine (pH 8.1), 330 mM sorbitol, 2.0 mM K_2-EDTA, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1.8 mM ADP, 0.9 mM Pi, 0.9 mM NADP, 6.8 μM Fd, and plastid lamellae equivalent to 12.9 μg Chl in the 7-h PLD and 13.4 μg Chl in 12-h PLD. All rates expressed are the average of two to three repetitions in the 1- to 5-min period of illumination. Twelve-h PLD were prepared and PS parameters measured from 0830 to 1000 hours; 7-h PLD were prepared and PS measured from 1230 to 1400 hours.

	Intact Plastids (CO_2-dependent O_2 evolution)	Plastid Lamellae (Fd, NADP, ADP, Pi)		
		O_2 evolution	NADP reduction	ATP synthesis
	μmol O_2 evolved/h·mg Chl	*μmol/h·mg Chl*		
Plastids from 7-h plants	91.6 ± 2.7	91.8 ± 10.4	200.8 ± 5.5	193.5 ± 7.3
Plastids from 12-h plants	55.2 ± 9.5	94.4 ± 14.0	203.7 ± 3.1	194.2 ± 1.4

Photo- and Dark Respiration. On a Chl basis, average PR rates were always approximately 2 times higher in the 7-h compared with the 12-h plant leaves (Table II). Dark respiration usually reflects mitochondrial CO_2 evolution, and it is now clear that mitochondrial respiration is functioning normally during leaf photosynthesis (1). On a Chl basis, 7-h plant leaves exhibited, at 15 d acclimation, approximately the same dark respiratory rate as the 12-h plants. Similar to photosynthetic rate, dark respiration rate, on a Chl basis, remained at nearly the same magnitude throughout the period of the 7-h day regime, but dark respiration in the 12-h plants declined by 30 to 50%.

Phosphorylated Intermediates in Leaves. The magnitude of foliar levels of RuBP, 3-PGA, GAP + DHAP, FBP, F6P, and GlP were monitored in 7- and 12-h daylength exposed plants from the point just prior to illumination (at 700 hours) and continuing through the illumination period (1010 and 1315 hours) (Fig. 1; 39-d-old plants described in Tables I and II). Correlated with the approximately 2-fold higher net foliar PS rate in the 7-h, relative to the 12-h plants, was a higher magnitude of change in the differential increase from dark to light in the level of RuBP, 3-PGA, and triose-P which was, respectively, 1.3-, 3.3-, and 2.5-fold greater in the 7-h compared with the 12-h plant foliage (Fig. 1, 700–1010 hours).

In the 7-h plant foliage, in response to the dark to light transition, FBP diminished from 37.3 to 32.8, but F6P rose from 0 to 21.5 nmol/mg Chl. Simultaneously, in the 12-h plant foliage, FBP rose from 19.1 to 37.7, but F6P decreased from 20.2 to 16.8 nmol/mg Chl. The greater magnitude of dark to light increase in F6P in the 7-h tissue suggested that in the light the phosphatative conversion of FBP to F6P was more rapid in the 7-h than in the 12-h tissue (Fig. 1).

Since RuBP is compartmentalized in the chloroplast (30), then the transient changes in the pool size of that intermediate in the leaf tissue reflected the actual dark to light concentration changes within the intact plastid *in vivo* (30). Levels of other intermediates, *e.g.* 3-PGA, triose-P, and hexose phosphates may reflect an average concentration of those moieties in several compartments, *e.g.* cytoplasm. However, the transient differential increase of these intermediates from dark to light reflected intact leaf chloroplast activity (Fig. 1) (30). These data (Fig. 1) are interpreted to mean that there was a greater Calvin cycle activity *in vivo* in the leaves of 7-h plants than there was in those of the 12-h plants.

Daylength Response and Isolated Chloroplast Photosynthesis. Spinach leaf chloroplasts isolated from 7-h plants (7-h PLD) which had been acclimated less than 12 d, usually displayed photosynthetic CO_2 assimilation rates as much as 40 to 50% lower compared with plastid preparations from 12-h control plants (12-h PLD). From the point of 12 d of 7-h daylength exposure forward to as much as 74 d, 7-h PLD had higher photosynthetic rates than 12-h plant isolates. Thirteen-d-adapted, 29-d-old 7-h plants, yielded PLD isolates which displayed rates of 69 to 70 μmol $^{14}CO_2$ incorporated/h·mg Chl while 12-h PLD preparations displayed rates of 63. Preparations isolated from 14-d-adapted plants displayed, in 7-h isolates, rates of 78 to 82, but in 12-h PLD rates were 64 to 67 (data not shown). By the point in time in which plants had been exposed to 7-h daylength for as much as 33 to 48 d, and since 12-h plant photosynthetic rates had declined during that period, 7-h PLD isolates often

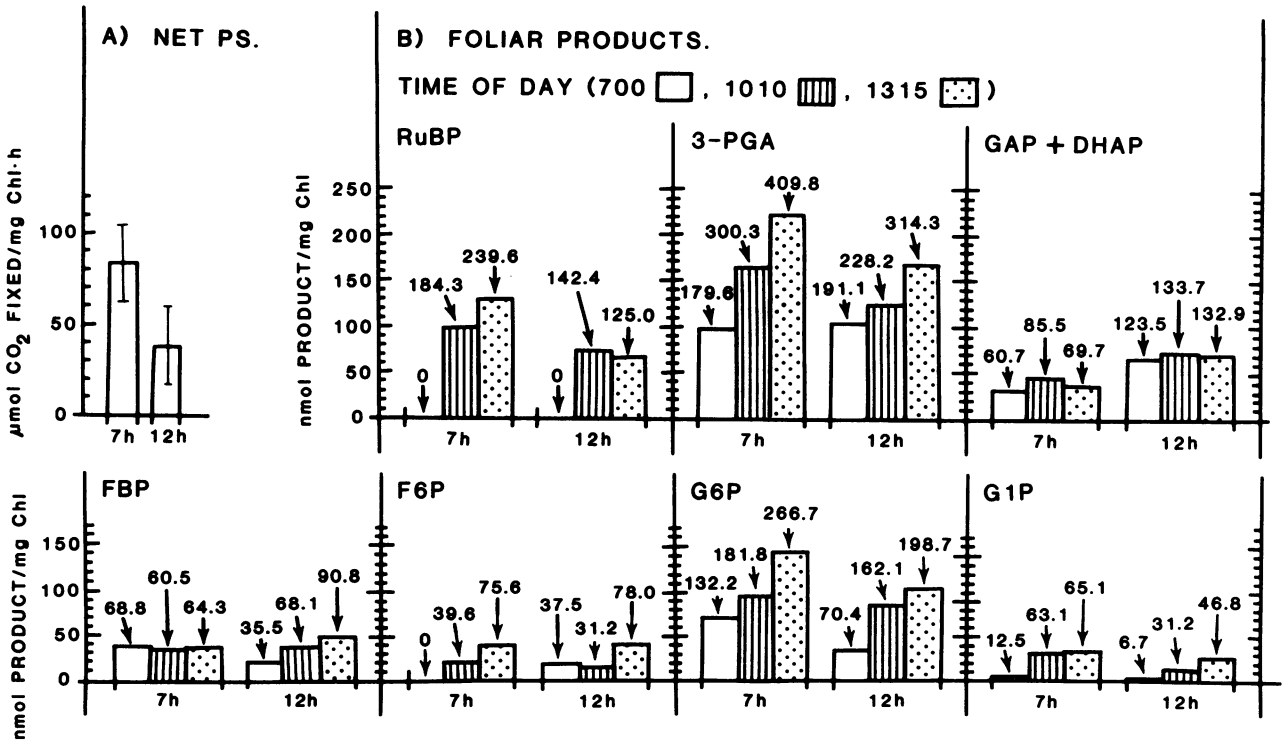

FIG. 1. Foliar level of phosphorylated intermediates in 7-h compared with 12-h daylength acclimated plants. Leaf tissues samples were from the same 39-d-old (29-d, 7-h regime and 39-d, 12-h control) described in Table I; illumination commenced at 700 hours for each 24-h cycle. Gas exchange measurements on single leaves were performed alternatively upon a 7-h plant and then a 12-h plant. Leaf sampling procedures are described in "Materials and Methods"; 5 to 10 mature leaves were sampled for each treatment at each time point. SLW values are reported in Table II; H_2O levels are recorded in the Figure 3 Legend. For purposes of comparison, intermediate levels are reported on a Chl basis (bars) as well as on a leaf area basis (values above the bars equal μmol product/ml·dm^2). Computation of average leaf intermediate level as a concentration in solution employed the same arithmetic relationships described for sucrose level in Figure 3. Measurement period for 7-h plants in light was extended to 1515 hours on the sampling day.

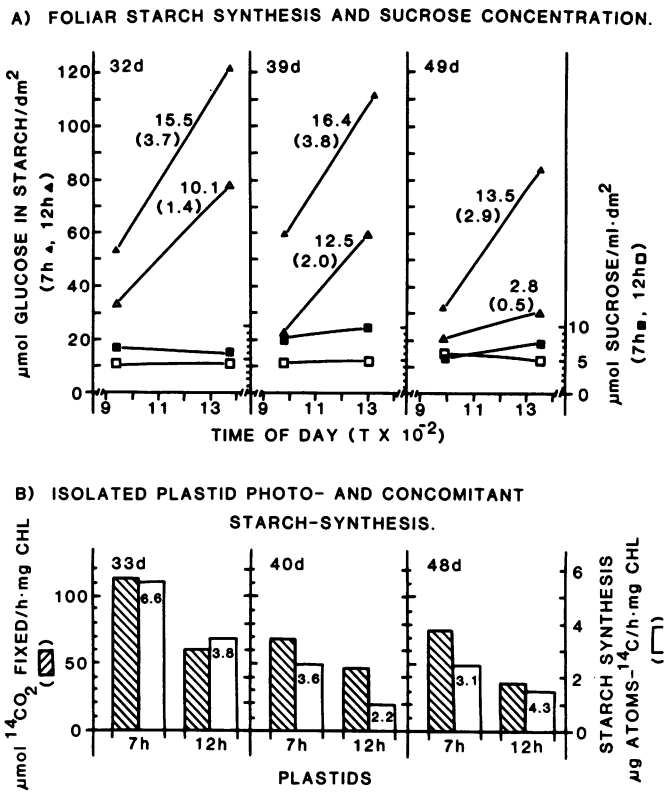

FIG. 2. Influence of daylength upon photosynthetic metabolism in progressively older spinach plants: A comparison of isolated intact chloroplast $^{14}CO_2$ photoassimilation and starch synthesis rate with foliar starch accumulation and sucrose level in the same plant populations from which the plastids had been isolated. Plants were propagated in the growth chambers from emergence on the 7- or 12-h light regimes described ("Materials and Methods"); daily illumination commenced at 700 hours. All data entries represent replicated determinations. Net PS rates were estimated only in the 49-d-old, 7- or 12-h plants, and the leaves of each acclimate displayed, respectively, average net PS rates of 57.3 ± 4.1 and 31.9 ± 2.2 μmol CO_2 fixed/mg Chl·h. A, Foliar starch and sucrose. (a) Average SLW for sampled leaves of 32-, 39-, and 49-d-old, 7- and 12-h plants was, respectively, 337 and 491 mg dry weight/dm² (930–1300 hours). (b) Average leaf Chl level for 7- and 12-h foliage was, respectively, 13.0 and 13.6 μg/mg dry weight. (c) Sucrose concentration was estimated in freeze-dried leaf tissue (see Fig. 3 for details). Seven- and 12-h plant foliage contained approximately 90% H_2O; 7- and 12-h plant leaves contained, respectively, 3.4 and 5.0 ml H_2O/dm². The numbers on the starch accumulation time course plot have the units μmol glucose incorporated/h·dm²; the parenthetical number is that rate as μmol/h·mg Chl. B, Isolated intact plastids. Measurements of plastid PS time course were made on the day following or preceding the day of leaf sampling. Photoassimilation of $^{14}CO_2$ was estimated in mixtures identical in composition with those described in Figure 4. Average Chl/reaction mixture and per cent intact plastids was for 7- and 12-h PLD, respectively, 21.3 μg and 59.2, and 24.3 μg and 60.5%. The numbers on the open bars represent the per cent of total ^{14}C incorporated into starch at the 20-min time point. Time of day designations: see Figure 5 legend.

displayed 1.5- to 2-fold higher rates of photosynthesis when compared with the 12-h PLD preparations (Figs. 2 and 4; Table III).

Daylength responses of PS provided a probe to ask whether or not the magnitude of NADPH and ATP photoproduction was a factor influencing CO_2 photoassimilation rates in 7- and 12-h isolates. CO_2-dependent, O_2 photoevolution was estimated, in the same experimental period, in preparations of 7- and 12-h PLD isolates (32-d-adapted) and plastid lamellae were prepared from the same 7- and 12-h PLD preparations and simultaneously tested for Fd-dependent O_2, ATP, and NADPH photoformation. As expected, CO_2-dependent, O_2 photoevolution in the intact plastid was approximately 40% higher in activity in the 7-h compared with the 12-h PLD preparations (Table III). In contrast, in the 7- and 12-h PLD plastid lamellae preparations, the magnitude of rates of O_2, ATP, and NADPH photoformation were not different (Table III). This implied that only the magnitude of the enzyme activities associated with photosynthetic carbon metabolism were factors in contributing to the higher rate of plastid CO_2 photoassimilation in the 7-h PLD isolates.

Intact plastids isolated from 40-d-old, 7- or 12-h acclimates were monitored for time course intermediate flow during $^{14}CO_2$ photoassimilation (Fig. 4; Table V). Intact plastid isolates (7- or 12-h PLD) from the daylength acclimates were incubated in identical reaction mixtures, *e.g.* 5 mM CO_2 and 0.25 mM Pi. Pi level was selected at that value, because that level supported both an optimal $^{14}CO_2$ fixation rate and an optimal starch formation rate in the preparations (29). Seven-h PLD displayed a 40% higher rate of $^{14}CO_2$ assimilation than the 12-h PLD, and this, in turn, resulted in a higher level of ^{14}C incorporated into phosphorylated intermediates formed with time (Fig. 4). Similar to the leaf tissue intermediate transients, ^{14}C-labeled RuBP (not shown), 3-PGA, GAP + DHAP, FBP, hexose P, glycolate (not shown), and starch all rose to almost twice the level in the 7-h compared with the 12-h PLD (Fig. 4).

Carbon-14 labeling patterns (partitioning) into intermediate pools during $^{14}CO_2$ photoassimilation in PLD isolates suggested that total Calvin cycle activity was greater in 7- than 12-h PLD isolates (Table V). P-glycerate and RuBP were initially higher in the per cent ^{14}C label in the 7- compared to the 12-h PLD, but labeling was not different compared in the two at 20 min.

Glyceraldehyde 3P + DHAP increased in the label over the total time course in the 7-h compared with the 12-h PLD, but FBP decreased in isotope labeling in the 7-h PLD. The hexose mono-P (F6P, G6P) were always lower in per cent ^{14}C label in the 7-h acclimate PLD, but starch labeling was higher; this suggested an enhanced flow of label from FBP to starch (Table V) as well as FBP metabolism to other Calvin cycle intermediates, *e.g.* RuBP.

Plastid Enzyme Activities. Plastid enzyme phosphate reductive cycle enzymes displayed higher activities in 7-h compared with 12-h PLD isolates. Plastid $^{14}CO_2$ photoassimilation was measured in intact plastid preparations from plants adapted to 33, 40, and 48 d to 7- or 12-h daylength; as previously noted, $^{14}CO_2$ photoassimilation rates were 1.5- to 2.0-fold greater in the 7-h PLD isolates (Figs. 2 and 4). The plastid CO_2 fixation rates diminished as the plants aged on both daylength regimes, but remained much higher in the 7-h isolates (Fig. 2). Simultaneously, with the estimation of plastid activities recorded in Figure 2 were concomitant measurements of the chloroplast enzymes fructose-1,6-bisP phosphatase, and ribulose-1,5-bisP carboxylase (Table IV). In plastid preparations from progressively older tissue, chloroplast fructose BisP phosphatase activity was always higher by a factor of 1.2 to 1.9 in the 7-h plastid isolate (compared with the 12-h). In all but the 40-d-old samples, activities of RuBP carboxylase were higher in the shortened day plastid. These enzymes displayed rates similar to the corresponding PLD CO_2 fixation rates (Fig. 2; Table IV).

Regardless of plant age and duration of shortened daylength adaptation, the activities of leaf plastid Calvin cycle enzymes remained significantly higher in isolates from the 7-h compared with the 12-h. In plastids prepared from leaves of 74-d-old spinach plants, adapted from emergence to a 7- or 12-h daylength enzyme activities were, for 7- and 12-h PLD isolates/h·mg Chl, respectively: (a) RuBP carboxylase, 372 and 326 μmol CO_2 fixed; (b) phosphoglycerate phosphokinase, 971 and 824 μmol 3-PGA

Table IV. *Chloroplast Enzyme Analysis Associated with Plastid Isolates Whose $^{14}CO_2$ Assimilation and Starch Synthesis Rates are Illustrated in Figure 2*

All enzyme activities were measured in the same experimental period and with the same plastid isolates described in Figure 2. Assay systems are described in "Materials and Methods." Average Chl level per assay for RuBP carboxylase and fructose-1,6-BisP phosphatase or ADPglucose pyrophosphorylase was, respectively: for 7-h PLD, 8.10 and 21.4; for the 12-h PLD, 12.0 and 24.0. Rates of enzyme activities are reported on the basis of 100% intact plastids. All entries represent the average of duplicate samples.

	Plant Age: Days Postemergence		
	33	40	48
(1) RuBP carboxylase—pH 8.1			
	μmol CO_2 fixed (RuBP-dependent)/h · mg Chl		
7-h PLD	185.9	62.8	85.4
12-h PLD	121.7	72.4	56.1
(2) Fructose BisP phosphatase—pH 8.1			
	μmol FBP converted to F6P/h·mg Chl		
7-h PLD	116.3	116.3	141.6
12-h PLD	94.7	94.2	82.3
(3) ADP glucose pyrophosphorylase—pH 8.1			
	μmol [U-^{14}C]ADP glucose formed/h·mg Chl		
7-h PLD	86.0	13.0	
12-h PLD	64.0	18.1	

reduced; (c) NADPH glyceraldehyde-3-P dehydrogenase, 737 and 468 μmol GAP formed; (d) fructose-1,6-bisP phosphatase, 147 and 115 μmol FBP converted to F6P; (e) transketolase, 532 and 310 μmol ribose-5-P converted to GAP; and (f) phosphoribulokinase, 261 and 242 μmol Ru5P converted to RuBP (data not shown; all rates are averages of duplicated assays). In this case, the most rate-limiting Calvin cycle enzyme appeared to be fructose-1,6-bisP phosphatase.

Starch Accumulation and Influencing Factors in Leaves and Isolated Plastids. I routinely observed, in leaves of spinach plants continuously exposed to 7-h daylength from emergence, an enhanced rate of glucose incorporation in leaf starch as well as the enhanced total level of starch (Fig. 2). Even as plants aged on the 7- and 12-h daylength regimes, starch synthesis trends remained apparent in the 7-h plants (Fig. 2). In that same study, 7-h PLD from 33- and 40-d-old plants reflected 1.6 to 1.7 times more ^{14}C label partitioned into the starch pool compared with the isotope labeling in 12-h PLD. An exception was observed in the PLD prepared from the 48-d-old plants in which per cent ^{14}C in starch was the same in both acclimate PLD (Fig. 2, 48 d). In that case, because the rate of CO_2 fixation was higher in the 7-h compared with the 12-h PLD, the absolute rate of starch synthesis was also higher in that case.

The data displayed in Figure 3 compared PS rate (dm^2 and Chl basis) (A) with foliar starch synthesis rate (B), as well as with the magnitude of partitioning of fixed CO_2 into starch (C) in 7-h compared with 12-h plant foliage. The 7-h plants displayed, on a Chl basis, a 1.2- to 2.1-fold higher average foliar plastid total PS rate than 12-h plant leaves, regardless of the magnitude of synthesis rate and level of starch. For example, at 39 d old, when 7-h plant PS rate was 2-fold higher than the 12-h plant, and when monitored from the end of the dark, the starch synthesis rate was 1.3 to 2.2 times higher in the 7-h compared with the 12-h plant foliage. In that sample, the daytime level of starch increased in the 7-h plants to approximately 1.1 times higher than it was in the 12-h plants. Foliar plastid PS rate was not inhibited by maximal partitioning of fixed CO_2 into starch. In the 25-d-old sample (Fig. 3C), in which there was, in first hours of illumination, a 5-fold preferential partitioning of fixed CO_2 into starch in 7-h compared with 12-h plant leaves, 7-h plant PS rate was not repressed when compared with 12-h plant PS rate (Fig. 3A).

At the 32- and 39-d-old sampling, the per cent of the total CO_2 fixed into starch was lower than, or equivalent, in 7-h compared with 12-h leaves (Fig. 3C). This diminished partitioning of photosynthate into starch in the 7-h plant foliage at these time points mirrored the improved growth rate of the 7-h plants (Fig. 3C; Table I).

In 7-h plant leaf tissue, the enhanced incorporation of glucose into starch was apparently not only a reflection of higher hexose monophosphate levels (Fig. 1), but in one case a higher total activity level of ADP-glucose pyrophosphorylase (Table IV). This enzyme has been identified as a limiting step in the synthesis of starch, and pyrophosphorylase activity is enhanced by an increased 3-PGA/Pi ratio (9, 18, 32). Pi in the chloroplast diminishes with time in the light due to Calvin cycle metabolism (11, 32). It was found in 7-h plant leaves, in which starch synthesis rate was higher than that of 12-h plant leaves, that there was often a 2-fold higher 3-PGA/Pi ratio when compared with 12-h plant leaves (Fig. 5). Because of the high background of level Pi, probably stored in vacuoles (8, 11), the ratio of PGA/Pi reported in Figure 5B was always less than 1.

Sucrose Accumulation in Leaves, Stems, and Roots. A repeated response of shortened daylength exposed plants was the accumulation of a higher level of sucrose in the 7-h, compared with the 12-h plant leaves. This trend was displayed on leaf population samples taken from spinach plants adapted to the 7-h daylength either after 10 d postemergence (Fig. 3), or, from those plants adapted from the emergence point (Fig. 1). Regardless of whether the photosynthetic rates were the same in 7-h compared with 12-h plants, or whether PS rates were higher in the 7-h plants, enhanced sucrose accumulation was observed (Fig. 3; Table II). For example, in the 15-d-adapted samples there was, in the 7-h plant foliage, a 5.5-fold increase in sucrose level during dark to light transition (700–945 hours) and a further increase was observed in the ensuing time interval (Fig. 3). In comparison, there was a 2.7-fold dark to light increase in the 12-h plant leaf sucrose level in the morning interval, with a slight increase in the afternoon (Fig. 3). This trend of sucrose increase was apparent as the plants aged on daylength regimes, except in the morning periods of the 39 to 49-d-old plants where sucrose level of both acclimates was approximately equivalent (Figs. 2 and 3).

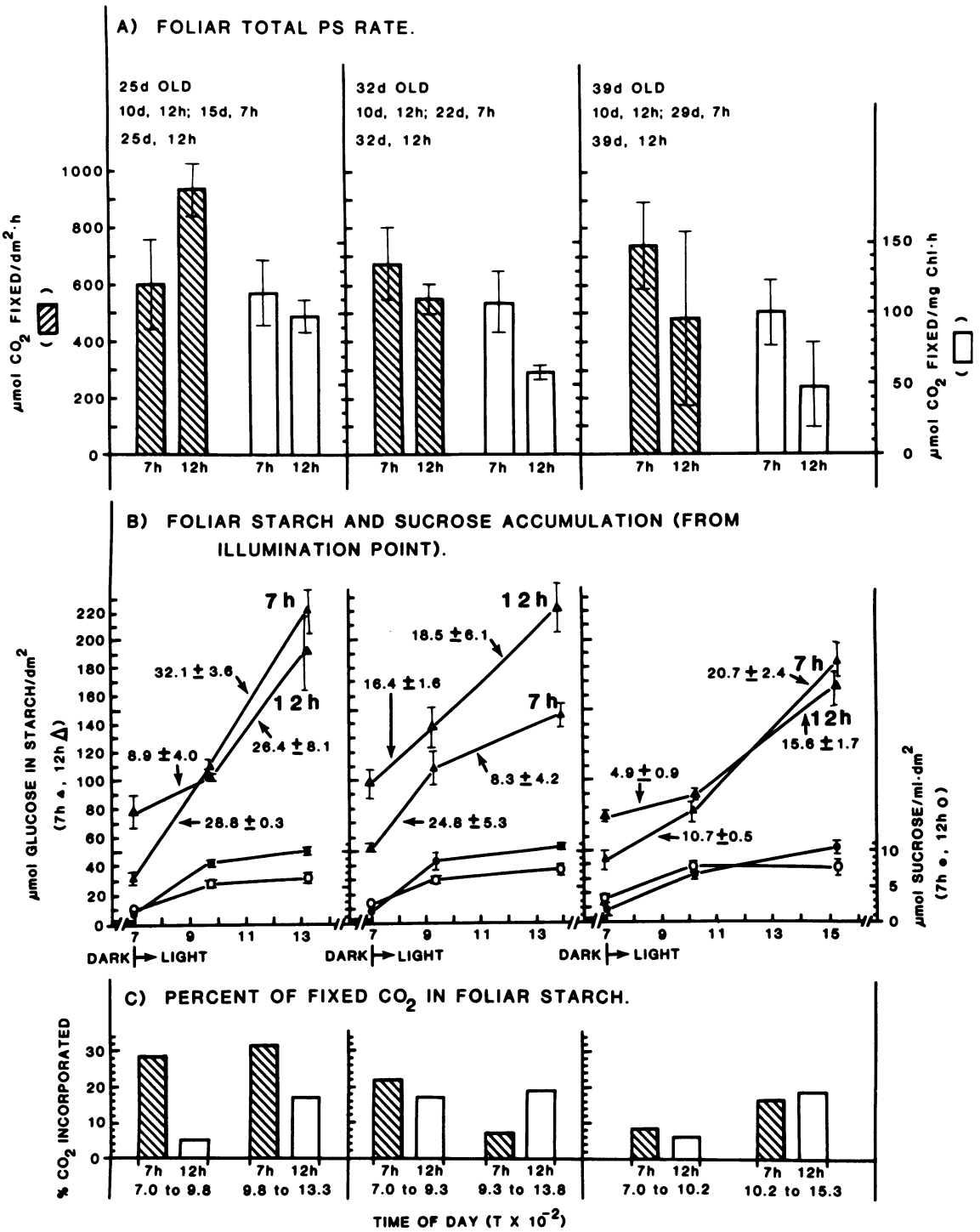

FIG. 3. Influence of shortened daylength upon sucrose and starch accumulation in leaves of progressively older spinach plants just prior to and during the illumination period. Plant tissues sampled were from the same treatment populations described in Table I and II; illumination commenced at 700 hours for both acclimates, and for the 12-h plants illumination ceased at 1900 hours; for the 7-h plants illumination ceased at 1400 hours except for the 39-d-old sample period where illumination was extended for 90 additional min. A, Total photosynthesis from Table II. B and C, Starch and per cent of the total CO_2 incorporated into that pool. This value was computed, for the morning and afternoon sampling periods for the leaves, by multiplying the average rate of starch synthesis in the defined periods by 6, and then dividing that resultant value by the rate of average total CO_2 assimilation (net PS + PR + DR) (from Table II). The resulting expression was multiplied by 100 to obtain the percentage. Example: for the AM period of the 25-d-old 7-h plants

$$\frac{(28.6\ \mu\text{mol glucose incorporated/dm}^2\cdot\text{h}) \times (6)}{(488.6 + 66.2 + 50.0 \text{ or } 605.2\ \mu\text{mol } CO_2 \text{ fixed/dm}^2\cdot\text{h})} \times 100 = 28.4\%\ CO_2$$

incorporated into starch during the interval. This calculation includes the assumption that all the glucose was derived from newly fixed CO_2 during the rate period, and additionally, it is assumed that each new molecule of glucose incorporated into starch was derived from 6 revolutions of the pentose phosphate reductive cycle. Mitochondrial respiration is assumed to be totally active during the illumination period (1). The numbers on the starch accumulation traces are the rates of glucose accumulation into starch as μmol/h·dm². B, Sucrose level. This compound is expressed as average μmol/ml in the tissue associated with 1 dm² of fresh tissue. This was computed by multiplying the SLW values reported in Table I by the measured μmol sucrose/mg dry weight and dividing the derived value by the ml H_2O computed to be present in one dm² of fresh leaf tissue. For 25-, 32-, and 39-d-old plant foliage, the ml H_2O/dm² for 7- or 12-h plants was, respectively, 4.2 and 7.6; 4.8 and 6.4; and, 4.0 and 5.4. Time of day designations: see Figure 5 legend.

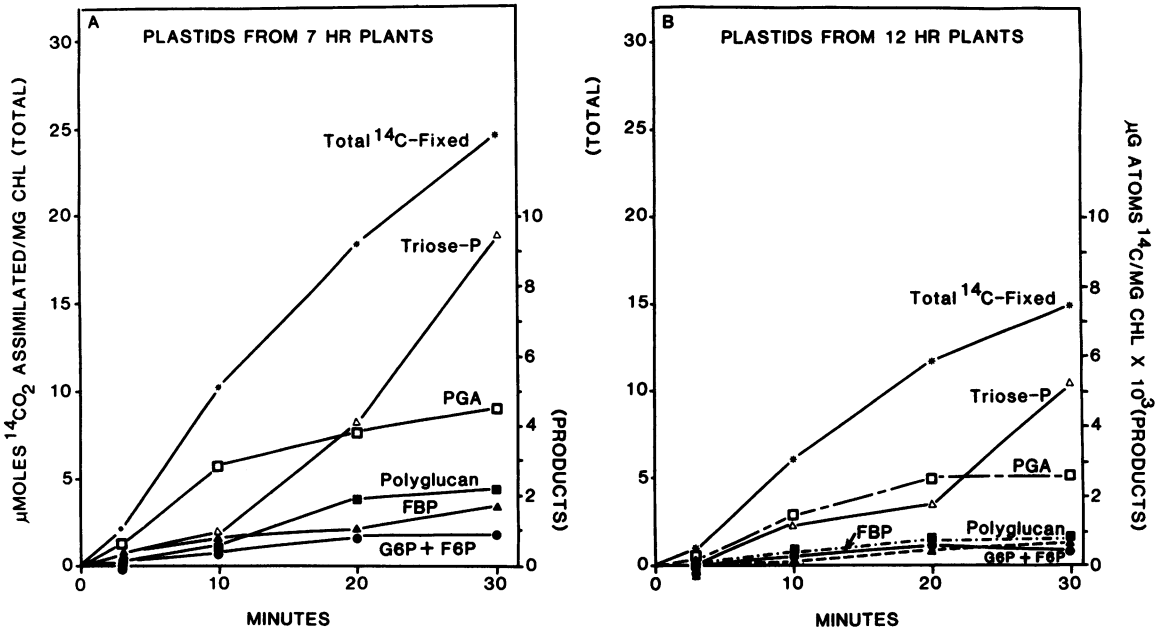

FIG. 4. Time course of light-dependent $^{14}CO_2$ incorporation into acid-stable intermediates in plastids isolated from the leaves of plants maintained on either a 12-h light/12-dark cycle or a 7-h light/7-h dark cycle. Plants, propagated from emergence on these daylength regimes, were 40 d old. The reaction mixtures contained, in 2.0 ml: 50 mM Tricine (pH 8.1), 330 mM sorbitol, 2 mM K_2EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.25 mM Pi, 5.17 mM ($NaH^{14}CO_3$ + $^{14}CO_2$) (1.934 μCi/μmol 'CO_2'), 1000 units catalase, and Chl for the 7-h or 12-h PLD was, respectively, 29.8 and 28.9 μg. Plastid intactness was in the 7-h PLD, 64.9%, and in the 12-h PLD, 73.9%. All reaction rates are corrected to a basis of 100% intact plastids. The 7-h PLD (A) displayed an average rate, in the 3- to 20-min period, of 56.8 μmol/h·mg Chl; the 12-h PLD (B) displayed an average rate of 36.8. Twelve-h PLD were isolated and PS measured 0915 to 1100 hours; 7-h PLD were isolated and PS measured from 1345 to 1530 hours.

I found that 12-h plant leaves appeared to be exporting more sucrose for metabolism than were the 7-h plant leaves, both in the light, and in the dark. For example, during the illumination period, the 32-d-old, 12-h plant stems contained approximately 25 to 29 mM, while 7-h plant stems only contained approximately 11.4 to 12.2 mM sucrose. Similarly, during illumination, tap roots of these same 12- and 7-h plant contained, respectively, 24.5 to 31.4 and 17.0 to 19.4 mM sucrose (data not shown).

Nocturnal Degradation of Starch and Sucrose. In comparison with the 12-h dark period of the longer day acclimate, the 17-h dark period of the 7-h dark regime provided a longer period for degradation and metabolism of starch and sucrose. This was evidenced by a much lower pool size of starch and sucrose at the end of the 17-h dark period compared with those levels at the end of the 12-h dark period (Fig. 3). Foliar sucrose level at the end of the dark period in 7-h plants, was routinely 42 to 58% lower than those levels measured in the 12-h acclimate leaves (Fig. 3). Since, in illuminated 7-h plant leaves, sucrose level was higher than in illuminated 12-h plant leaves, then there was a greater magnitude of nocturnal sucrose disappearance in the 7-h leaves (Fig. 3).

DISCUSSION

Plant Growth. Adaptation by spinach plants to a 7-h compared with a 12-h daily duration of PS involved several changes, not only in plant growth (Table I), but also in PS, PR, and DR (Figs. 1 and 4; Tables II and III), as well as in carbon metabolism (Figs. 1 to 5; Tables IV and V). Chatterton and Silvius (3) found that soybean plant growth rapidly adapted (4 d) to a 7-h daylength. In contrast, it required at least 13 to 15 d of 7-h daylength exposure before spinach plant growth began to increase (Table I). During the initial 13 to 15 d of 7-h daylength adaptation, growth was severely repressed, but in the subsequent period (15–29 d) growth increased dramatically. During this same period, 12-h plants had already accrued a much greater leaf area and thickness (Tables I and II), and their photosynthate requirements for growth were satisfied, because there was a longer daily duration of PS and there was more Chl/dm^2 (Tables I and II). In the 12-h plants, the magnitude of the total photosynthate production or 'source strength' was sufficient such that although PS rate diminished, further growth was well supported (Table I). After 15 d of 7-h daylength exposure, plants were in a state of increased growth. Because there was less leaf area with thinner leaves and less Chl/dm^2 (Tables I and II), the requirement for photosynthate exerted by plant growth caused there to be a sustained higher rate of PS from the 13- to 15-d point onward.

Photosynthetic Rate Determination. The increased and sustained 7-h plant PS rates, compared with the decreased PS rates in 12-h plants, provided a probe to examine metabolic factors that exert influence on the magnitude of PS (Table II). Since the magnitude of ATP and NADPH photoproduction was the same when compared in 7- and 12-h PLD-lamellae preparations, and since the isolated intact plastid CO_2 photoassimilation was greater in magnitude in the 7-h PLD, it was concluded that photosynthetic electron transport properties were not rate-determining to total PS in the 7- and 12-h plant leaves (Table III).

Although the mechanisms for communication between sink organs and source leaf chloroplasts remain to be defined (8, 11), at least one of the factors that established the magnitude of PS rate in 7- and 12-h plants was the magnitude of activity of several pentose phosphate reductive cycle enzymes. In the 7-h, compared with the 12-h PLD, it was clear that the increased PS rates were attributable to higher magnitudes of activities of not only RuBP carboxylase (16, 31), but also FBP phosphatase as well as other enzymes in that system (Table IV; "Results"). That Calvin cycle activity was higher in the foliage of 7-h, relative to 12-h plants, was evidenced by the greater magnitude, in the 7-h leaves, in the change of dark to light transient levels of intermediates including RuBP, 3-PGA, triose-P, F6P, and GlP (Fig. 1). Isolated intact Chl $^{14}CO_2$ photoassimilate transients and ^{14}C labeling trends in

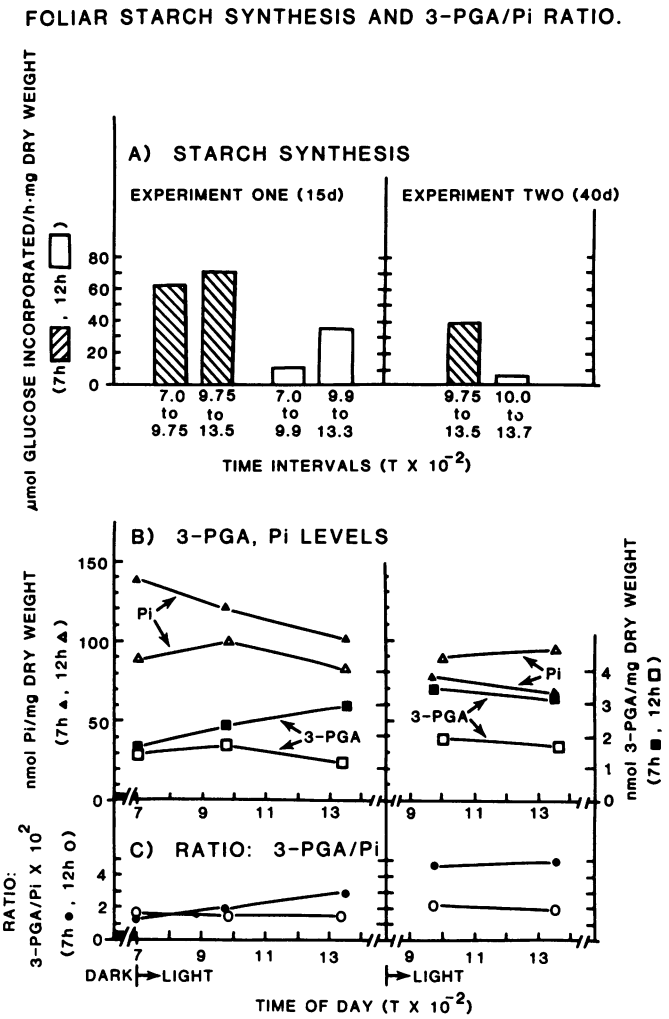

FIG. 5. Rate of starch synthesis, level of Pi and 3-PGA, and 3-PGA/Pi ratio in leaf tissue of spinach plants acclimated to a 7- or 12-h daylength. Experiment 1: plants were taken from the 25-d-old group described in Table I. Experiment 2: plants were taken from the 40-d-old group described in Figure 2. Time of day designations: In the part A plot, fractions of 1 h are based on 100 divisions/h, *e.g.* at 15 d, 7.0 to 9.75 h is equivalent to $(7.0\text{–}9.75 \times 10^2) \times (10^{-2})$, and this designates 700 to 945 h which is based on 60 min/h.

intermediate pools also reflected a greater Calvin cycle activity in the 7-h, compared with the 12-h PLD (Fig. 4; Table V).

That chloroplastic and cytoplasmic interrelationships such as the Pi-triose-P exchange between those compartments influence the PS and starch synthesis rate in isolated plastids and isolated leaf mesophyll cells has been demonstrated repeatedly (8, 11, 29, 30, 32). The results of these daylength response experiments do not deny the influence of cytoplasmic-chloroplastic relationships in potential regulation of PS rate. On the other hand, these studies (Figs. 2 and 4; Tables II–IV) indicated that PS rate is established, in response to demand for photosynthate during plant development, and that removal of the chloroplast from its cellular environment does not remove this expression of the magnitude of PS activity. Indeed, once the magnitude of Calvin cycle activity was established *in vivo*, then 7- and 12-h PLD, removed from their parent tissues, still reflected that magnitude of the foliar PS rate even though the PLD reaction mixture components were identical (Figs. 2 and 4; Table III–V).

Photosynthetic Rate and Sucrose-Starch Levels. Clearly, the magnitude of PS, when expressed on a Chl basis, had no correlation with foliar starch and sucrose accumulation level and rate (Fig. 3). Even when 7-h plant foliage reflected the highest starch accumulation rate and level (at 15 d adaptation) there was never repressed 7-h plants PS rate (Fig. 3). Along with higher PS rate, the magnitude of increase of foliar sucrose level during the dark to light transition in the 7-h plants was often nearly twice that displayed by the 12-h plant leaves (Figs. 2 and 3). I must agree with the most recent arguments which refute the notion that enhanced starch and sucrose level results in a 'feedback' inhibition to reduce photosynthetic rate (8, 10, 11). It is conceivable that higher levels of sucrose, starch, or photosynthetic intermediates could, in fact, lead to an increase in Calvin cycle activity (19, 24). Certainly, if there is a demand for photosynthate, then, regardless of the magnitude of accumulation of sucrose and starch, there will be an increased photosynthetic rate, and that is the case in the plants adapted to 7-h daylength (Fig. 3; Tables I and II) (3, 5).

Table V. *Per cent ^{14}C Incorporated into Photosynthetic Intermediates as a Function of Time for the 7- and 12-h PLD Study in Figure 4*

Products	% ^{14}C in Products at Following Illumination Times			
	Plastids from 7-h plants		Plastids from 12-h plants	
	3 min	20 min	3 min	20 min
Products				
Polyglucan (starch)	2.2	10.3	1.2	6.1
RuBP	2.8	0.5	0	0.4
Fru-1,6-bisP	14.0	5.6	7.1	5.1
Glu-6-P + Fru-6-P	4.4	4.7	10.7	6.3
Rib-5-P	3.2	3.7	7.1	6.1
Triose-P	13.2	22.3	21.2	15.6
3-P-glycerate	28.5	20.6	22.7	21.6
Glycolate	1.0	1.3	1.2	1.4
Unknown	30.7	31.0	28.8	37.4
Total %	100	100	100	100

In part, the enhanced accumulation of sucrose in 7-h plant leaves was apparently facilitated by a restriction of export of sucrose from the foliage ("Results"). Work in several laboratories (3, 5, 10, 27) has indicated that photosynthate export from 'source' leaves of shortened daylength-adapted plants was very restricted compared with that of 12-h plant leaves. Furthermore, it was conceivable that sucrose accumulation in the 7-h plant leaves was a reflection of a preferential loading of sucrose into vacuoles rather than into the phloem for export (8, 27).

Factors Affecting Starch Synthesis Rate. At all stages of growth examined, the leaves of 7-h plants usually displayed a higher rate of starch synthesis than 12-h plants, although there were some exceptions (Figs. 2 and 3). Starch synthesis rates and the magnitudes of the partitioning of fixed CO_2 (the per cent total CO_2 incorporated) into starch were always greatest in the earliest stages of 7-h daylength exposure, and this was a reflection of slowest growth in these initial periods (Fig. 3; Table I). Preferential allocation of photoassimilate into starch in source leaves is often a reflection of decreased sink organ development (8, 17), and conversely, increased photosynthate demand in sink organs (growth) often results in diminution of accumulation of source leaf starch (8, 31). As growth in the 7-h plants progressed, the per cent of total CO_2 fixed in starch in the 7- and 12-h plant leaves became equivalent, and this reflected the improved growth in the 7-h test plants from the 15th to the 39th d of exposure (Fig. 3). However, even when there was reduced partitioning of photosynthate into starch in 7-h plants, there was usually a higher starch synthesis rate, and this also implied that synthesis rate was associated with the higher 7 h plant PS rate (Fig. 3).

When compared with 12-h PLD isolates 7-h PLD preparations reflected higher starch synthesis rates and, in some cases, a greater magnitude of partitioning of photosynthate into that polymer pool (Fig. 2) even though both 7- and 12-h PLD isolates were assayed in reaction mixtures of equivalent composition (Figs. 1 and 4). That suggested that control of preferential partitioning of photoassimilate to starch resided within, and not outside of the chloroplast. Rate enhancement of starch synthesis appeared to be associated with factors in the chloroplast that affected the activities of chloroplast ADP-glucose pyrophosphorylase.

Rate determination in the plastid starch synthesis pathway may be exerted at the level of the enzyme ADP-glucose pyrophosphorylase (9, 18, 29, 32). In one case, in 7-h daylength plants, where there was observed a higher percentage (partitioning) of fixed CO_2 allocated to starch, there was observed a higher specific activity of ADP-glucose pyrophosphorylase in the 7-h compared with the 12-h PLD (when both activities were assayed with 1 mM 3-PGA and 0.25 mM Pi) (Fig. 1B; Table IV, 33 d/7-h compared with 12-h PLD). I concluded that, when starch synthesis rates and photosynthate allocation into starch were at highest magnitudes (Fig. 3C, 15 d, 7 h), a control point for starch synthesis was an increased magnitude of total activity of the pyrophosphorylase.

Starch synthesis rate in 7-h plant leaves was correlated with an increased 3-PGA/Pi ratio (Fig. 5), a situation which also would enhance the *in vivo* activity of the plastid ADP-glucose pyrophosphorylase, and which would result in a more rapid starch synthesis (9, 18, 32). This was associated with a higher rate of PS in 7-h plant leaves and PLD preparations, because the magnitude of increase in 3-PGA was greater in the 7-h plant plastids and leaves (Figs. 1, 4, and 5), and because the increased PS rate is accompanied by a more rapid diminution of Pi level due to a more rapid utilization of Pi in the chloroplasts (11, 32).

Finally, the K_m for G1P for spinach leaf ADP-glucose pyrophosphorylase in the presence of 3-PGA has been estimated to be 40 μM (18). In the 7-h plant foliage, the average level of G1P was 63 to 65 μM, but in 12-h foliage it was 31 to 47 μM (Fig. 1). Based on this observation, I concluded that the level of G1P was more rate-limiting to the phosphorylase in the 12-h plant leaves, than it was in the 7-h plant foliage. Thus, the level of that hexose monophosphate also contributed to the higher rate of starch synthesis in the 7-h compared with the 12-h plants (Figs. 1 and 3, 39-d-old with 29 d at 7 h).

Dark and Photo-Respiration. It was certainly clear that mitochondrial respiration played a role in the overall adaptation of the spinach plants in adjusting to growth and daylength regime, because the DR rate also remained high during the periods of improved growth (Tables I and II). Coupled with the greater magnitude of nocturnal disappearance of sucrose in the 7-h relative to the 12-h plant leaves (Fig. 3B), high 7-h plant DR rates reflected the partially successful effort to compensate for a limited daily acquisition of photosynthate in terms of meeting energy and carbon skeleton demands during the longer night.

Photorespiratory rate was higher in the 7-h leaves (Table II) and this may have been a reflection of higher glycolate production, because of increased photosynthate level (19). In some experiments, I found that the percentage of ^{14}C in glycolate, incorporated during ^{14}CO assimilation in plastid isolates, was greater in 7-h PLD (18.5%) than in 12-h PLD (15.8%). Again, since the 7-h PLD $^{14}CO_2$ photoassimilatory rates were greater than the 12-h PLD, then glycolate photosynthesis was greater in magnitude. Higher glycolate production may have led to some enhanced sacrifice of photosynthate by peroxisomal activity. On the other hand, higher peroxisomal metabolism could have contributed additional amino acids as a supplement to those produced elsewhere in the 7-h plant leaf tissue.

Acknowledgments—I thank D. R. Lee, D. F. Kremer, K. Yu, K. Englund-Harris, W. Stracke, P. Romas, and W. A. Dungey for many hours of technical assistance, and S. S. Kravitz and J. D. Cusack for their patience and help in manuscript preparations.

LITERATURE CITED

1. AZCON-BIETO J, CB OSMOND 1983 Relationship between photosynthesis and respiration. The effect of carbohydrate status on the rate of CO_2 production by respiration in darkened and illuminated wheat leaves. Plant Physiol 71: 574–581
2. BAHR JT, RG JENSEN 1978 Activation of ribulose bisphosphate carboxylase in intact chloroplasts by CO_2 and light. Arch Biochem Biophys 185: 39–48
3. CHATTERTON NJ, JE SILVIUS 1979 Photosynthate partitioning into starch in soybean leaves. I. Effects of photoperiod *versus* photosynthetic period duration. Plant Physiol 64: 749–753
4. CHATTERTON NJ, JE SILVIUS 1981 Photosynthate partitioning into starch in soybean leaves. II. Irradiance level and daily photosynthetic period duration effects. Plant Physiol 67: 257–260
5. CHATTERTON NJ, JE SILVIUS 1980 Acclimation of photosynthate partitioning and photosynthetic rates to changes in length of the daily photosynthetic period. Ann Bot 46: 739–745
6. DICKSON RE 1979 Analytical procedures for the sequential extraction of ^{14}C-labeled constituents from leaves, bark, and wood of cottonwood plants. Physiol Plant 45: 480–488
7. FISKE CH, Y SUBBAROW 1925 The colorime.ric determination of phosphorus. J Biol Chem 66: 375–400
8. GEIGER DR, RT GIAQUINTA 1982 Translocation of photosynthate. *In* Govindjee, ed, Photosynthesis, Vol 2. Academic Press, New York, pp 345–386
9. GHOSH HP, J PREISS 1966 Adenosine diphosphate glucose pyrophosphorylase. A regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J Biol Chem 241: 4491–4504
10. GORDON AJ, GJA RYLE, DF MITCHELL, CE POWELL 1982 The dynamics of carbon supply from leaves of barley plants grown in long or short days. J Exp Bot 33: 241–250
11. HEROLD A 1980 Regulation of photosynthesis by sink activity—the missing link. New Phytol 86: 131–144
12. HUBER SC, DW ISRAEL 1982 Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. Plant Physiol 69: 691–696
13. KELLY GJ, G ZIMMERMANN, E LATZKO 1976 Light induced activation of fructose-1,6-bisphosphatase in isolated intact chloroplasts. Biochem Biophys Res Commun 70: 193–199
14. LATZKO E, M GIBBS 1968 Distribution and activity of enzymes of the reductive pentose phosphate cycle in spinach leaves and in chloroplasts isolated by different methods. Z Pflanzenphysiol 59: 184–194
15. LATZKO E, M GIBBS 1974 Measurement of the intermediates of the photosynthetic carbon reduction cycle using enzymatic methods. Methods Enzymol 24: 261–268
16. LILLEY R MCC, DA WALKER 1974 An improved spectrophotometric assay for ribulose bisphosphate carboxylase. Biochim Biophys Acta 358: 226–229
17. MONDAL MH, WA BRUN, ML BRENNER 1978 Effects of sink removal on photosynthesis and senescence in leaves of soybean (*Glycine max* L.) plants. Plant Physiol 61: 394–397
18. RIBEREAU-GAYON G, J PREISS 1971 ADP-glucose pyrophosphorylase from spinach leaf. Methods Enzymol 23: 618–624
19. ROBINSON JM, M GIBBS 1974 Photosynthetic intermediates, the Warburg effect, and glycolate synthesis in isolated spinach chloroplasts. Plant Physiol 53: 790–797
20. ROBINSON JM, MG SMITH, M GIBBS 1980 Influence of hydrogen peroxide upon carbon dioxide photoassimilation in the spinach chloroplast. I. Hydrogen peroxide generated by broken chloroplasts in an "intact" chloroplast preparation is a causal agent of the Warburg effect. Plant Physiol 65: 755–759
21. ROBINSON JM, RC SICHER, NJ CHATTERTON 1980 Influence of photosynthetic duration upon rate of photosynthesis and starch synthesis in spinach leaves and plastids. Plant Physiol 65: S-71
22. ROBINSON JM, DF KREMER, K ENGLUND 1981 Influence of photosynthetic period upon metabolic conditions affecting starch synthesis in spinach leaves and plastids. Plant Physiol 67: S-36
23. ROBINSON JM, M GIBBS 1982 Hydrogen peroxide synthesis in isolated spinach chloroplast lamellae. An analysis of the Mehler reaction in the presence of NADP reduction and ATP formation. Plant Physiol 70: 1249–1254
24. SCHACTER B, JH ELEY, M GIBBS 1971 Involvement of photosynthetic carbon reduction cycle intermediates in CO_2 fixation and O_2 evolution by isolated chloroplasts. Plant Physiol 48: 707–711
25. SERVAITES JC, WL OGREN 1977 Rapid isolation of mesophyll cells from leaves of soybean for photosynthetic studies. Plant Physiol 59: 587–590
26. SHEN L, J PREISS 1964 The activation and inhibition of bacterial adenosine diphosphoglucose pyrophosphorylase. Biochem Biophys Res Commun 17: 424–429
27. SICHER RC, WH HARRIS, DF KREMER, NJ CHATTERTON 1982 Effects of shortened daylength upon translocation and starch accumulation by maize, wheat, and pangola grass. Can J Bot 60: 1304–1309
28. SILVIUS JE, DF KREMER, DR LEE 1978 Carbon assimilation and translocation

in soybean leaves at different stages of development. Plant Physiol 62: 54–58
29. STEUP M, DG PEAVEY, M GIBBS 1976 The regulation of starch metabolism by inorganic phosphate. Biochem Biophys Res Commun 72: 1554–1561
30. STITT M, W WIRTZ, HW HELDT 1980 Metabolite levels during induction in the chloroplast and extrachloroplast compartments of spinach protoplasts. Biochim Biophys Acta 593: 85–102
31. THORNE JH, HR KOLLER 1974 Influence of assimilate demand on photosynthesis, diffusive resistances, translocation, and carbohydrate levels of soybean leaves. Plant Physiol 54: 201–207
32. WALKER DA 1976 Regulatory mechanisms in photosynthetic carbon metabolism. Curr Top Cell Regul 11: 203–241
33. WOLF DD, RB PEARCE, GE CARLSON, DR LEE 1969 Measuring photosynthesis of attached leaves with air sealed chambers. Crop Sci 9: 24–27