CARBON DIOXIDE FIXATION BY ETIOLATED PLANTS AFTER EXPOSURE TO WHITE LIGHT[1,2]

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Upon exposure of an etiolated plant to light, photochemical, biochemical, and physiological changes occur. Total chlorophyll synthesis and the conversion of protochlorophyll to chlorophyll a, have been studied extensively (10, 13). Gross physiological changes and cessation of elongation have been the subject of many investigations in plant physiology. Some of these processes are independent of chlorophyll synthesis and photosynthesis (22). A few changes in enzymatic activity during the greening of an etiolated plant have been reported (1, 8, 17).

Measurements of the formation of chlorophyll by the etiolated plant after exposure to light have been extensively investigated. Oxygen evolution by etiolated barley plants after they were placed in the light indicated that this process did not commence until the seedlings had been in the light 30 minutes (14). Experiments with etiolated oats also showed oxygen evolution after chlorophyll formation (6). Irving (9) reported that greening etiolated barley and bean seedlings did not fix CO_2 until they had developed a large part of their chlorophyll. Observations have now been made on the fixation of $C^{14}O_2$ and the products formed during increasing time intervals after an etiolated wheat plant has been placed in the light. The results indicate that for many hours after the start of illumination, there is a slow and irregular formation of some of the reactions in CO_2 fixation during photosynthesis.

PROCEDURE

Etiolated Thatcher wheat plants were grown at 24° C in sand with Hoagland nutrient solution. On the seventh day the plants were illuminated with between 600 and 1000 fc of continuous white light from 150-watt reflector flood bulbs. Water filters removed the heat. Fifteen leaves were taken for chlorophyll analyses (22) at hourly intervals after illumination began. At the same intervals but with different batches of wheat, the ability of the leaves to fix CO_2 was measured. For this latter test, one or two leaves were exposed in a small-scale photosynthesis chamber (7) for 10 minutes at 1000 fc of light to an atmosphere of air containing $C^{14}O_2$. The volume of the chamber was 100 ml into which was released 25 μc of C^{14} from $NaC^{14}O_3$ of a specific activity of 25 % C^{14} so that the final CO_2 concentration did not increase appreciably. During this period the temperature was kept at 23° C by submerging the apparatus in a water bath.

At the end of the 10-minute period, the C^{14}-exposed leaves were killed by maceration in boiling 30 % methanol. An aliquot was counted for total C^{14} fixation in the soluble extract. Another portion was subjected to paper chromatographic analysis, water-saturated phenol being used as the first solvent and butanol–propionic acid–water for the second development (3). Autoradiographs were made of each chromatogram, with No-Screen x-ray film to locate the radioactive compounds; the total counts per spot were determined with a G-M tube. This activity was then expressed as a percentage of the total activity on the chromatogram or as actual counts per second of the compound in one leaf. Since it was not practicable to start with an exact quantitative aliquot on each paper chromatogram, counts of radioactivity in the compounds among different experiments may be in error by as much as 15 %. The percentage distribution of radioactivity within one experiment should not be more variable than the counting errors.

In later experiments etiolated plants were exposed to light after first being sprayed with various compounds which might effect the greening process. In separate experiments, plants were sprayed with water, 0.3 M glucose, 0.3 M ribose, 0.1 M mixture of 20 % sedoheptulose and 80 % sedoheptulosan (designated sedoheptulose after its physiologically active component), or 0.1 M glycolic acid. The plants of each experiment were sprayed, while still in the dark, at 24, 22, 19, 16, and 0 hour intervals before illumination. Bottles for spraying indicator solutions on paper chromatograms were used. These conditions were chosen since the same procedure with glycolic acid has been shown to activate glycolic acid oxidase (17). Glycolic acid at pH 2.3 was used since these previous experiments have shown that the sodium salt of the acid when sprayed on leaves was not effective in activating glycolic acid oxidase. After exposure to light and just before the 10-minute photosynthesis test with $C^{14}O_2$, the leaf blades were cut off and washed briefly under running, distilled water to remove excess reagent. The leaves appeared normal and contained no visible bacterial or mold contamination from this treatment.

RESULTS AND DISCUSSION

Several light intensities were used in trial experiments to ascertain the most advantageous conditions for chlorophyll synthesis. These qualitative results indicated that light intensities around 1000 fc produce the most rapid greening; whereas, in very high light intensity from a photoflood bulb or sunlight, the plants greened very slowly. Of equal importance, etiolated leaves cut off at their base and placed in water turned green and fixed $C^{14}O_2$ at much slower rates than in experiments where the plant remained

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intact during the greening period. Racusen and Aronoff have shown that decapitated leaves incorporate amino acids into protein more slowly than in the intact plant (11), and thus the slower greening process in decapitated etiolated leaves in the present experiments may reflect less rapid protein synthesis for the formation of the chloroplast. However, cutting off the leaf for the final 10-minute photosynthesis experiment with $C^{14}O_2$ did not seem to affect the $C^{14}O_2$ fixation. Thus there are several differences between the present experiments and those of Irving (9): 1) The use of the whole etiolated plant rather than a cut-off top has been found necessary for satisfactory results; 2) more controlled and somewhat higher light intensities have been used; and 3) radioactive $C^{14}O_2$ has permitted measurements of rate of fixation and products formed. It has not been possible to ascertain the effect of unlabeled CO_2 produced inside the cell by respiration on the rate of fixation of external radioactive $C^{14}O_2$. However, the absence of C^{14} in the products of the path of carbon in photosynthesis for several hours of greening would indicate no photosynthetic fixation, and permits qualitative measurements on the formation of this process.

Chlorophyll Formation and CO_2 Fixation Rates: Figure 1 shows chlorophyll content and total $C^{14}O_2$ fixation at various times after etiolated leaves were first exposed to light. A very small amount of chlorophyll was present after the plants had been in the light for 1 hour, owing to the rapid light-catalyzed conversion of protochlorophyll to chlorophyll, but large amounts were not produced for 2 to 3 hours. The rate of chlorophyll production was most rapid from about the third to the twelfth hour of light. After this period, the amount of chlorophyll in the plant increased more slowly, on wet weight basis, for about 20 hours. After 1.5 days of continuous light, the now fully green plant contained a normal amount of chlorophyll.

The rate of $C^{14}O_2$ fixation was very slow for about 1 to 2 hours after rapid chlorophyll production had started, i.e., after about 5 hours of continuous light. The amount fixed before this time, as measured accurately by the isotope method, amounted to less than

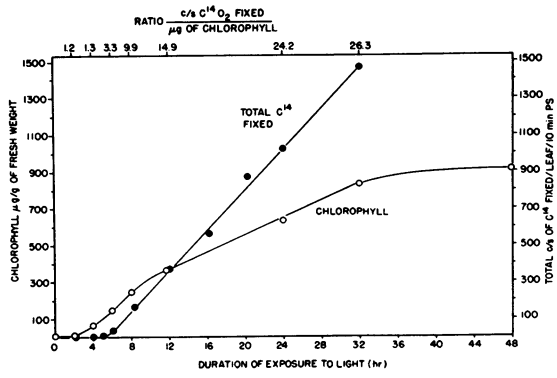

Fig. 1. Total chlorophyll and rate of $C^{14}O_2$ fixation during greening.

0.1 % of that fixed by the leaves after greening. Thus when these etiolated plants were placed in the light, several hours were required for the rate of chlorophyll synthesis to become significant, and then 1 to 2 more hours of light before photosynthesis commenced, as measured by $C^{14}O_2$ fixation. Total fixation of $C^{14}O_2$ began to increase at a rapid, linear rate after photosynthetic fixation began, around the fifth hour of light, and so continued to increase during the next 24 hours or longer. After about 32 hours the plant was fully green and fixed CO_2 at a rate equal to that of a normal plant. Almost all the C^{14} was fixed into the soluble extract during the 10-minute photosynthesis test period with $C^{14}O_2$ by the greening plants and the data reported are values for the activity in the soluble fraction only.

Smith (14) noted that oxygen evolution during greening in etiolated barley seedlings began within 30 minutes which indicated that a Hill reaction was functioning in the whole leaves. At that time the chlorophyll present was chiefly the small amounts formed by the initial photochemical conversion of protochlorophyll to chlorophyll. Evolution of oxygen from oat leaves soon after exposure to light has also been reported (6). CO_2 fixation, however, appears to commence later if comparison between these different monocotyledonous plants is reasonable. Such a comparison seems valid in that the rate and time of chlorophyll synthesis were similar.

The ratio of the rate of $C^{14}O_2$ fixation to total chlorophyll is given across the top of figure 1 for each period of analysis after the etiolated plants were placed in the light. Data on amount of chlorophyll formed has been reported as microgram per gram of fresh tissue weight, in conformance with similar figures in the literature. The average weight of one leaf (< 100 mg) for each analysis was determined for calculating this ratio, since the leaves for $C^{14}O_2$ fixation experiments were not weighed, in order to facilitate rapid killing for subsequent chromatographic analysis of the products. The ratios indicate that with increasing time in 1000 fc of light the chlorophyll present in the greening wheat became much more efficient in promoting CO_2 fixation. This ratio, calculated for these particular experimental conditions, and C^{14} specific activity could vary with changes in light intensity, temperature, and CO_2 partial pressure. Increasing light intensity and CO_2 pressure might tend to increase the difference in this ratio since the very small fixation rate in the slightly green plant should not be limited by these factors. However, further studies based on Blackman's principle of a rate-limiting step in the multiple process of photosynthesis should aid in determining which portions of photosynthesis are rate limiting during various times in the greening process.

Products of $C^{14}O_2$ Fixation: The products formed by fixation of $C^{14}O_2$ for a 10-minute period at the end of each hour after the plants had been placed in white light were analyzed by paper chromatography. Figures 2 A and B are autoradiographs of

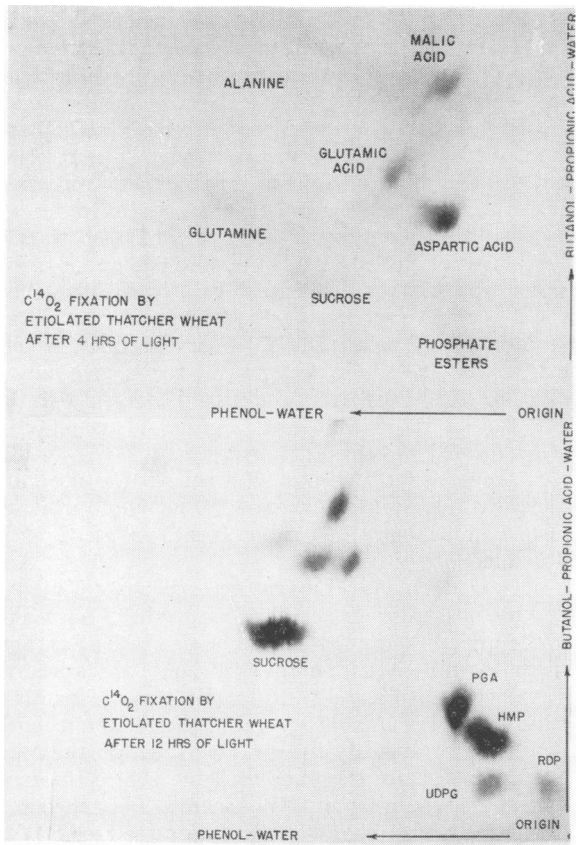

FIG. 2. Products from $C^{14}O_2$ fixation: A (*above*), after 4 hrs of light; B (*below*), after 12 hrs of light.

the chromatograms of leaves which had had 4 hours and 12 hours of light, respectively. C^{14} activity in each compound was counted after detection on the chromatograms by autoradiography, and is expressed in figure 3 as a percentage of the total C^{14} fixed during that period. During the first 4 hours after the

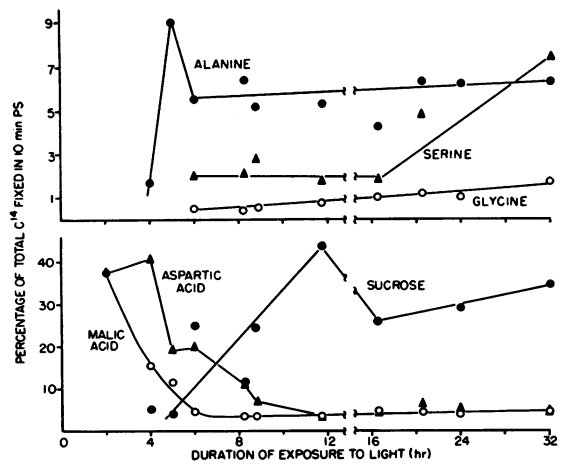

FIG. 3. Percentage of total C^{14} fixed in certain compounds with increasing lumination periods.

etiolated plants were placed in the light, the only three products to be labeled by $C^{14}O_2$ in significant amounts were malic, aspartic, and glutamic acids. The curve for percentage of C^{14} in glutamic acid (not shown) was similar to that for aspartic acid; however, there was only about one-half as much label in glutamic acid. These products represent respiratory fixation. As will be shown later, small amounts of C^{14} in phosphoglyceric acid (PGA) and sugar phosphates were also detectable by the isotope method after 4 hours of light. But the amount of this photosynthetic fixation was not significant even though the plants were in the light and had formed appreciable amounts of chlorophyll by this time.

As the plant greened during 2 to 8 hours of light, the total C^{14} incorporated into malic, aspartic, and glutamic acids increased manyfold as chlorophyll was produced (table I). The great increase in the amount of total C^{14} in the respiratory products was perhaps stimulated by the photosynthetic reducing power be-

TABLE I

RELATIVE AMOUNTS OF C^{14} INCORPORATED IN MALIC, ASPARTIC, AND GLUTAMIC ACIDS

DURATION OF LIGHT *	TOTAL FIXATION PER LEAF PER 10 MIN		
	MALIC	ASPARTIC	GLUTAMIC
hr	*c/s*	*c/s*	*c/s*
2	>1	>1	<1
4	4	10	3
6	7	29	6
8	22	71	31
9	54	97	45
12	56	50	38
24	164	232	158
32	268	334	46

* For chlorophyll concentration at each of these periods, see figure 1.

ing produced by the newly formed chlorophyll. The early increase was probably not caused by an increase in the pool size of newly formed intermediate compounds in the photosynthetic cycle, since these compounds had not yet accumulated in appreciable amounts.

The first product, other than the respiratory acids, to become labeled with C^{14} after the etiolated wheat plants had been placed in the light was a small amount of PGA after about 4 hours of light (tables IV, V, VI). At the fifth hour, PGA and alanine contained an abnormally high percentage of the total C^{14} fixed. Figure 3 shows the percentage of C^{14} in alanine at this time. The C^{14} activity in alanine was probably derived from PGA through pyruvic acid, though the intermediate C_3 compounds did not accumulate in appreciable amounts. It appears that the plants, at this stage of greening, were able to reduce little of the PGA to the aldehyde level and then convert it to carbohydrate. With plants exposed to white light for longer periods of time, the percentage of the total C^{14} fixed in the alanine dropped to a smaller, but there-

after constant, amount. However, the total radioactivity in the C_3 compounds continued to increase with increasing rate of $C^{14}O_2$ fixation.

These results suggest that after 5 hours of light, the plant was able to commence slow photosynthetic carboxylation to form PGA, of which part was converted to alanine. Several explanations are then possible to explain the accumulation of alanine and PGA, with little C^{14} activity appearing in the sugars. At that stage, the plant may not have had sufficient reducing power or active enzymes to reduce rapidly PGA to hexose. On the other hand, saturation of the alanine and PGA pools may have been necessary for equilibria favoring synthesis of sucrose. In either case, this condition seemed to exist for an interval of 1 to 2 hours at about the fifth hour after etiolated Thatcher wheat had been placed in white light.

After exposure of the plant to light for longer than 5 hours, quantities of C^{14} began to appear in the sugar phosphates and sucrose. The percentage of C^{14} going into sucrose and sugar phosphates, as reflected by sucrose (fig 3), increased rapidly at this time, to reach a high and relatively constant maximum percentage value after the plants had been illuminated for 9 to 12 hours. Longer periods of greening seemed to have no further effect on the percentage of the fixed C^{14} which appeared in sucrose. However, it is important to note that after this period of light, the total C^{14} fixed was still only 15 to 20 % of that of a fully greened plant after 32 hours of light (fig 1). The data indicate that by this time the reactions in the reduction of PGA were functioning at about the equilibrium conditions of normal steady-state photosynthesis. The increase in total fixation rate after 12 hours must be the result of the increasing rate of other, more slowly developing processes; such as formation of chlorophyll and of the CO_2 acceptor, which is ribulose diphosphate (RDP) or related to it. On the basis of the increasing chlorophyll efficiency ratio for total CO_2 fixation with further greening, still other factors probably exist which limit photosynthesis after only 12 hours of greening.

Formation of Serine and Glycine: Serine and glycine acquired C^{14} from $C^{14}O_2$ during greening of the etiolated plant on a different time sequence from that of alanine (fig 3). PGA and alanine became radioactive during the 10-minute $C^{14}O_2$ fixation period in leaves that had greened for 4 and 5 hours but glycine and serine were not labeled. In fact, about 20 hours of light were required for a large amount of C^{14} to be incorporated into serine. During photosynthetic fixation experiments with $C^{14}O_2$ by green barley or wheat plants, glycine and serine are always labeled with a large portion of the C^{14} fixed (5, 16). It has been assumed that these two amino acids were labeled quickly during short-time photosynthesis experiments because they were end products of a short side reaction from the carbon cycle of photosynthetic fixation (12, 18, 20, 21). At least two pathways have been proposed for glycine and serine formation in plants; namely, the formation of serine from PGA, or their formation from glycolic and glyoxylic acids. Glycolic acid is readily converted to glycine and serine in plants (12, 18) and should arise from the two carbon fragments by the action of transketolase on the sugar phosphates formed during photosynthesis. Furthermore, it has been shown that a key enzyme, glycolic acid oxidase, of this side reaction is activated in etiolated plants during the greening process in order to establish this pathway (17). For Thatcher wheat, this activation period required about 20 hours of light. In the present experiments, again with Thatcher wheat, a rapid movement of C^{14} into serine was also not established until after 20 hours of light, which implies that the major pathway for the in vivo incorporation of C^{14} into serine during photosynthesis in this plant is via glycolic acid. Serine did not appear to be formed directly from triose and PGA, as has been proposed for soybeans (20), since it was not substantially labeled during the first 20 hours of greening, while alanine and PGA were being produced with large amounts of C^{14}.

CO_2 Fixation by Leaves Sprayed with Various Compounds: A more detailed analysis has been made of the organic phosphate esters of the path of carbon in photosynthesis during the first hours the etiolated plant acquired the ability to fix $C^{14}O_2$ photosynthetically. Also, amounts of several of these intermediates were raised artificially by spraying them on the etiolated plant before exposure to light. Since the percentage incorporation of C^{14} in sucrose had not varied significantly after the plants had been in the light for 9 to 12 hours, in these experiments data on plants in the light for longer than 8 hours were not kept except for one 25-hour exposure.

Total C^{14} fixed by approximately equal leaf samples sprayed with water in the dark 24 hours (used as controls) did not vary significantly from that fixed by unsprayed plants (table II). Feeding glucose to the etiolated plant prior to the experiment gave some increase in the rate of chlorophyll synthesis but a sub-

Table II

Influence of Spraying Various Substrates on Etiolated Wheat Leaves Prior to Illumination on Rate of Photosynthesis

Duration of light	Solution sprayed on leaves prior to light exposure				
	Water *	Glucose	Ribose	Sedoheptulose	Glycolic acid
hr	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*
2	13	4	8	3	10
3	8	48	75	4	12
4	54	92	350	11	23
5	103	274	253	57	151
6	238	230	172	180	374
7	300	527	303	290	250
8	725	510	478	370	550
25	1042	1800	1471	1350	1400

* Total C^{14} fixed during a 10-min test period with $C^{14}O_2$ into the water soluble extract from 2 leaves.

stantially higher rate of CO_2 fixation during the critical time after 3 to 5 hours of light. Ribose fed to the etiolated plant stimulated CO_2 fixation even more. For both sugars, there still remained a 2-hour lag in light before CO_2 fixation began. In both cases, but not for the water-sprayed control, after 6 hours of light there was a great falter in the rate of increase of CO_2 fixation by the plant as if some precursor or factor had been exhausted, and its synthesis was rate limiting for a few hours thereafter. One explanation for this may be that the presence of the free sugars in large amounts in the leaf resulted in an unusual demand on adenosine triphosphate (ATP) for their phosphorylation before they could be used for the plant's growth. Thus much of the ATP produced by the photosynthesis process (15) may not have been available at this time for regeneration of the intermediates for photosynthesis.

Sedoheptulose sprayed on the etiolated leaves in the dark inhibited CO_2 fixation rather than stimulating it. These results were not expected since sedoheptulose phosphate is intimately involved in the metabolic processes of photosynthesis (4). Since 80 % of this sugar was present in the spray as its anhydride, and since this anhydride is not metabolized by barley leaves (19), there is a possibility that the inhibition of the path of carbon of photosynthesis during greening may be due to this unnatural form of the seven carbon sugar.

Glycolic acid had no effect on the rate of CO_2 fixation during the first hours the leaves were in the light. Glycolic acid, when applied in exactly the same manner, has been shown to activate glycolic acid oxidase in this plant (17). Therefore, this enzyme does not appear to be rate limiting in photosynthesis during greening, but rather is a side reaction from this process.

After illumination for about 1 day, all the plants sprayed with the various compounds had a higher rate of $C^{14}O_2$ fixation than the water-sprayed control. Compounds sprayed on the plant were then perhaps being utilized to stimulate growth of the plant.

In table III are tabulated the amounts of radioactive carbon fixed in sucrose and the organic phosphate esters located on the chromatograms between PGA and the origin. These compounds represent a major fraction of the total C^{14} fixed and include all the sugar phosphates and PGA which are the active constituents of the path of carbon in photosynthesis. Glucose-, and especially ribose-treated plants, had a stimulated sucrose synthesis after 4 hours and again after 25 hours of illumination, in comparison to the water controls. The data indicate that the increased total fixation (table II) was a result of photosynthesis and not of greatly enhanced respiratory fixation.

Composition of Organic Phosphate Esters: The first product of photosynthetic $C^{14}O_2$ fixation is carboxyl-labeled PGA and the immediate precursor for this carboxylation is probably the pentose sugar, ribulose diphosphate (2). In the greening, etiolated leaf, the pool size of this precursor should be vanishingly small or nonexistent. Analysis of the plant tissue by the chromatographic solvents employed gave poor separation of the phosphate esters. These areas on the chromatogram have been designated according to their major component as PGA, HMP (hexose monophosphate), UDPG (uridine diphosphate–glucose), and RDP (fig 2 B). A more detailed analysis of these phosphate ester areas has been made by eluting each phosphate area separately and hydrolyzing the ester with a crude phosphatase preparation (Polidase S). Rechromatography of the organic moiety containing the C^{14} gave good separation and provided identification of the original phosphate esters in each area. In tables IV, V, and VI, the yields of such dephosphorylated products have been given relative scores to indicate the order in which these key esters acquire C^{14} from $C^{14}O_2$ during the greening period.

Results in table IV are for the Thatcher wheat

TABLE III

Sucrose and Organic Phosphate Esters Formed During Photosynthesis by Greening Wheat Leaves

Duration of light	Reagent sprayed on leaves before experiment									
	Water		Glucose		Ribose		Sedoheptulose		Glycolic acid	
	Sucrose	Phosphates *	Sucrose	Phosphates	Sucrose	Phosphates	Sucrose	Phosphates	Sucrose	Phosphates
hr	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*	*c/s*
2	Trace	<1	0	Trace	Trace	1	0	Trace	Trace	<1
3	Trace	<1	4	8	6	14	Trace	Trace	<1	<1
4	4	10	8	13	..	..	1	3	2	4
5	8	25	42	79	42	84	4	13	20	34
6	50	54	46	80	36	37	11	31	38	88
7	42	68	94	157	34	67	33	61	49	56
8	140	131	136	181	88	112	33	77	107	120
25	217	143	705	337	601	302	334	138	443	296

* Phosphates include the C^{14} in PGA, HMP, UDPG, and RDP. A more detailed analysis of these areas is given in tables IV, V and VI.

TABLE IV

COMPOSITION OF PHOSPHATE ESTERS FORMED DURING 10-MIN PHOTOSYNTHESIS BY ETIOLATED WHEAT LEAVES AFTER 24-HR SPRAYING WITH WATER AND THEN EXPOSURE TO WHITE LIGHT

DURATION OF LIGHT AND PHOSPHATE AREAS	HYDROLYSIS PRODUCTS *					
	GLYCERIC ACID	GLUCOSE	FRUCTOSE	SEDOHEPTULOSE	RIBULOSE	RIBOSE
2 hr						
Total area	Trace	Trace	0	0	0	0
3 hr						
PGA	+	0	0	0	0	0
HMP	0	Trace	Trace	0	0	0
UDPG ...	0	Trace	0	0	0	0
4 hr						
PGA	++	Trace	Trace	0	0	0
HMP	Trace	++	+	Trace	0	0
UDPG ...	0	+	0	0	0	0
RDP	0	Trace	Trace	Trace	0	0
5 hr						
PGA	++	Trace	Trace	0	0	0
HMP	Trace	++	+	Trace	0	0
UDPG ...	0	+	0	0	0	0
RDP	0	Trace	+	Trace	Trace	Trace
6 hr						
PGA	+++	Trace	0	0	0	0
HMP	Trace	++	+	Trace	0	0
UDPG ...	0	+	0	0	0	0
RDP	0	Trace	+	Trace	Trace	Trace
25 hr						
PGA	+++++	Trace	+	0	0	0
HMP	+	+++	++	++	0	0
UDPG ...	0	++	0	0	0	0
RDP	Trace	Trace	Trace	Trace	Trace	Trace

* The C^{14} labeled PGA, HMP, UDPG and RDP areas each were eluted separately from the original paper chromatogram, enzymatically hydrolyzed with Polidase S, the products rechromatographed, and autoradiograms made of new chromatograms. These autoradiographs were scored by visual observation while comparing whole sets of chromatograms. The total C^{14} in the combined organic phosphate areas before hydrolysis are given in table III.

leaves that had been sprayed with water and serve as controls for tables V and VI. After the etiolated plants had been in the light for 3 hours, detectable C^{14} activity was present in PGA, though this was but a small percentage of the total fixed into respiratory products. A vanishingly small trace of activity (too small to count) could be detected by autoradiography in glucose and fructose from the HMP and UDPG areas. The results indicate that the PGA pool had built up first. After 4 hours of light the plant can reduce a little $C^{14}O_2$ to the hexose sugar phosphates but the predominant fixation was still into malic, aspartic, and glutamic acids. The appearance of C^{14} in the hexoses indicates some reversal of the Embden-Meyerhof pathway at this stage.

The 10-minute exposure of $C^{14}O_2$ used in these experiments would be considered so long a labeling period in photosynthesis with a fully green leaf that about uniform distribution of C^{14} in the carbon atoms of all the sugars would be expected. Therefore the appearance of significant amounts of C^{14} in PGA alone over a 10-minute period represents a tremendous change in rate.

Only traces of C^{14} activity were present in RDP from the 5th- to the 25th-hour analyses after illumination of the leaves. Sedoheptulose phosphate did not appear to accumulate significant amounts of label until almost a day after the etiolated plants were placed in the light. This suggests that one reason for the lag in CO_2 fixation during the greening period and for the low efficiency of the newly formed chlorophyll was the failure of the plant to drive the photosynthetic cycle at a rate sufficiently high to regenerate enough RDP. The reversal of the Embden-Meyerhof pathway seemed to have been functioning satisfactorily, probably because many of its enzymes had been involved in the growth of the etiolated seedling.

It is impossible to determine by these data alone whether the absence of active enzymes in the regeneration of RDP may have been responsible for slow recycling of the C^{14} to ribulose diphosphate. The very short lag phase in CO_2 fixation (seconds to a few minutes) by a green plant, when transferred from dark to light, should be compared to the lag in photosynthesis of over 24 hours during the greening of the etiolated plant. The length of the induction period in photosynthesis by a fully green plant may be a measure of the rapidity with which the plant can

TABLE V

COMPOSITION OF PHOSPHATE ESTERS FORMED DURING 10-MIN PHOTOSYNTHESIS BY ETIOLATED WHEAT LEAVES AFTER 24-HR SPRAYING WITH GLUCOSE AND THEN EXPOSURE TO WHITE LIGHT

DURATION OF LIGHT AND PHOSPHATE AREAS	HYDROLYSIS PRODUCTS					
	GLYCERIC ACID	GLUCOSE	FRUCTOSE	SEDOHEPTULOSE	RIBULOSE	RIBOSE
2 hr						
Total areas	Trace	0	0	0	0	0
3 hr						
PGA	++	Trace	+	0	0	0
HMP	Trace	++	+	Trace	0	0
UDPG ...	0	+	Trace	Trace	0	0
4 hr						
PGA	+++	0	Trace	0	0	0
HMP	Trace	++	+	Trace	0	0
UDPG ...	0	++	Trace	Trace	0	0
5 hr						
PGA	+++++	Trace	Trace	0	0	0
HMP	+	+++	++	+	0	0
UDPG and RDP ...	Trace	++	Trace	Trace	Trace	Trace
6 hr						
PGA	+++++	Trace	Trace	0	0	0
HMP	+	+++	++	+	0	0
UDPG ...	0	++	Trace	0	0	0
RDP	0	Trace	Trace	Trace	+	Trace
25 hr						
PGA	++++	Trace	Trace	0	0	0
HMP	+	+++	++	+	0	0
UDPG ...	Trace	+++	0	0	0	0
RDP	Trace	++	Trace	Trace	++	+

TABLE VI

COMPOSITION OF PHOSPHATE ESTERS FORMED DURING 10-MIN PHOTOSYNTHESIS BY ETIOLATED WHEAT LEAVES AFTER 24-HR SPRAYING WITH RIBOSE AND THEN EXPOSURE TO WHITE LIGHT

DURATION OF LIGHT AND PHOSPHATE AREA	HYDROLYSIS PRODUCTS					
	GLYCERIC ACID	GLUCOSE	FRUCTOSE	SEDOHEPTULOSE	RIBULOSE	RIBOSE
2 hr						
PGA	+	+	Trace	0	0	0
UDPG ...	0	Trace	0	0	0	0
3 hr						
PGA	++	Trace	+	Trace	0	0
HMP	Trace	++	+	+	0	0
UDPG ...	0	+	Trace	0	0	0
RDP	0	Trace	Trace	Trace	0	0
4 hr						
PGA	++	Trace	+	Trace	0	0
HMP	Trace	++	+	Trace	0	0
UDPG and RDP ...	0	+	0	0	0	0
5 hr						
PGA	+++	0	0	0	0	0
HMP	+	+++	++	+	0	0
UDPG and RDP ...	Trace	++	+	Trace	Trace	0
6 hr						
PGA	+++	0	0	0	0	0
HMP	0	++	++	+	0	0
UDPG ...	0	+	0	0	0	0
RDP	0	Trace	+	Trace	Trace	Trace
25 hr						
PGA	+++++	+	++	+	0	0
HMP	++	+++++	+	++	0	0
UDPG ...	Trace	++	0	0	0	0
RDP	Trace	+	Trace	Trace	++	+

build up the pool size of carbon compounds in photosynthesis provided all the enzymatic processes are present. The 24-hour lag in photosynthesis and production of significant RDP by a greening etiolated plant, therefore, can hardly be explained by the slow accumulation of significant pools of these compounds. Rather, it would appear that synthesis of adequate amounts of enzymes to catalyze some of these reactions might be a limiting factor.

In the area of the sugar phosphate esters there was one unknown with an R_f of 0.2 in both the solvents used in chromatography. This unknown was not hydrolyzed by Polidase S. Its pool size with respect to total C^{14} became constant after the etiolated leaves had had only 4 hours of light. Thus the rate of labeling of this unknown was like that for PGA and alanine at the fifth hour of greening, except that it was present in rather small amounts and as the plant greened further, more total C^{14} was not incorporated into the compound. In these respects it was unique among the compounds being labeled by the $C^{14}O_2$.

When the etiolated leaves were sprayed with glucose prior to illumination, the rate and amount of $C^{14}O_2$ incorporation into PGA and the hexose were stimulated (table V). However, detectable label in pentose phosphates did not occur in the leaves until after they had been exposed to the same long period of light as required by the water-sprayed controls. Leaves sprayed with ribose prior to the light exposure had an even faster and more diversified buildup of the organic phosphate esters of photosynthesis (table VI). In these leaves, there were measurable amounts of PGA and hexose phosphates after only 2 hours of light; after 3 hours of light, considerable sedoheptulose phosphate was present, although it did not appear with the water controls until the fifth hour, and not in this amount until the twelfth hour of light. Nevertheless, RDP still showed the same small pool size at these times. This may be caused by some limiting step unaffected by the excess ribose present; or the large pool of fed and unlabeled pentose may prevent, by mass action, rapid recycling of the fixed $C^{14}O_2$ through RDP. This would have resulted in all the newly formed C^{14}-labeled PGA being used for hexoses and sucrose synthesis. However, after the plants had been in the light for 1 day, substantial RDP was present, which probably reflects the stimulated total CO_2 fixation by plants fed this sugar.

In table VII are summarized the relative amounts of C^{14} appearing during the 10-minute photosynthesis period with $C^{14}O_2$ in the products of an etiolated Thatcher wheat plant after exposure to 3 hours of light. At this time, C^{14} label could be detected in the first photosynthetic fixation products, although the amount fixed in them was not an appreciable percentage of the total fixation. Prior spray feeding of either glucose or ribose solutions to the etiolated leaves in the dark stimulated C^{14} fixation into PGA, glucose and fructose phosphates, and sucrose, but not into RDP.

Since the unique carboxylation reaction of photosynthesis utilizes RDP, the production of C^{14}-labeled PGA, hexoses, and sucrose without producing detectable label in the pentoses or RDP during the early phases of the greening process indicate that the pentoses were present in exceedingly small and rate-limiting amounts. As expected, feeding ribose to the etiolated plant resulted in a large increase in photosynthetic fixation at these times, since the plant should be able to convert it to RDP. In an etiolated plant not

TABLE VII

RELATIVE AMOUNTS OF C^{14}-LABELED PRODUCTS FROM 10-MIN PHOTOSYNTHESIS BY ETIOLATED WHEAT AFTER EXPOSURE TO 3 HR OF LIGHT

PRODUCTS	SOLUTIONS SPRAYED ON LEAVES PRIOR TO LIGHT EXPOSURE				
	WATER	GLUCOSE	RIBOSE	SEDOHEPTULOSE-SEDOSAN	GLYCOLIC ACID
Aspartic acid .	+++	++++	++++	+++	+++
Malic acid ...	++	+++	+++	++	++
PGA area	+	++	++	0	+
HMP area ...	Trace	+++	++++	Trace	+
UDPG area ..	Trace	+	+	0	Trace
Sucrose	Trace	+++	+++	Trace	+

sprayed with ribose, other sources for the RDP must be available for the first carboxylations, such as from the alternate glucose metabolism pathway. Afterward, the pool size of RDP remained very small, with the result that none had been detected in these experiments. An explanation has not been found for the formation of a large pool of hexoses and sucrose by the etiolated plants after 12 hours in the light while not converting them to RDP. At this time the plants were quite green, but the total photosynthetic CO_2 fixation was still relatively low. One explanation which needs further testing is that of slow enzymatic adaptation for RDP synthesis.

Summary

Etiolated Thatcher wheat plants were exposed to 1000 fc of white light and at hourly intervals afterward determinations were made of total chlorophyll, rate of $C^{14}O_2$ fixation, and the C^{14} products which were formed. Continuous, rapid chlorophyll synthesis began after 2 to 3 hours of light. During the first 4 hours of light, C^{14} was fixed at a low rate into malic, aspartic, and glutamic acids. After 4 and 5 hours of light, PGA and alanine were labeled, and after 5 to 6 hours of light C^{14} appeared in hexose phosphates and sucrose at an increasing rate. At the beginning of photosynthetic fixation there was a period during which C^{14} accumulated in PGA and alanine without being further reduced to hexoses in large amounts. After 9 to 12 hours of light, the percentage of the total C^{14} fixed which was incorporated among PGA, sucrose, and hexose phosphates, was constant, but only at one-quarter the rate of a normally green plant.

A limiting factor during this greening process was the availability of RDP, which may have been partially limited by slow enzymatic adaptation in the plant. The effect on the subsequent greening process of spraying glucose, ribose, sedoheptulose-sedoheptulosan, and glycolic acid on the etiolated plants in the dark prior to the exposure to light was studied. Ribose, and to a lesser extent, glucose, stimulated CO_2 fixation during the first hours of greening, but the amount of RDP present still appeared to be a rate-limiting factor. Glycolic acid was without effect and sedoheptulose-sedoheptulosan was inhibitory to the formation of an active photosynthetic cycle.

Label did not appear in large amounts in serine until after the period for activation of glycolic acid oxidase. This indicated that the major pathway for serine synthesis during photosynthesis was via the glycolic acid pathway.

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PHOSPHORUS AND SULFUR COMPOUNDS IN PLANT XYLEM SAP[1]

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Since radioactive phosphorus and sulfur have become available, numerous investigators have determined the rate and conditions for the movement of these two essential materials from the soil through the roots to the leaves. The work has involved detection of the radioactivity as a measure of phosphorus or sulfur movement. It has been assumed that inorganic phosphate and sulfate were the major if not the only forms in which these elements moved in the upward-flowing xylem of the plant stem. Reports on phosphorus compounds in this plant sap are conflicting; Pierre and Pohlman (4) reported some phosphorus present as organic esters, while Lundegårdh (3) found no organic phosphates in the exudation of roots. The present investigation with radioactive tracers and paper chromatographic analyses indicates that, though inorganic phosphate is a major form of phosphorus being transported, there are in the xylem sap two other phosphorus-containing compounds one of which may account for a substantial portion of the transported phosphorus. Inorganic sulfate appears to be the only form of sulfur being transported.

PROCEDURES

Tests have been made with plants which produce a relatively large amount of exudate (bleeding) when their stems or leaf bases were cut off. Sacramento barley has been used in most of the experiments. The plants were grown in sand or soil in the greenhouse or in Hoagland's nutrient solution in a constant environment room with about 800 fc of light on a 16-hour day and at 75 to 77° F. In some experiments, the plants in sand or soil have been used as grown; in others, the sand or soil was carefully washed off the roots before placing them in 0.1 or 0.01 strength Hoagland's nutrient solution during the experiment to which P^{32} or S^{35} was added. Plants grown in sand or soil into which P^{32} phosphate was poured or injected yielded poorer results than those grown in aerated nutrient solution with P^{32}. The sand or soil bound the phosphate so tightly that the rate of appearance of P^{32} in the plant stem was more erratic. Flooding the sand or soil with the solution containing the tracer caused poor exudation. Polyethylene beakers were used for holding the P^{32} nutrient solution since the radioactive phosphate did not adhere tightly to the side wall of these containers. Plants were suspended by glass grids or by corks.

The radioactive phosphate or sulfate was placed in the dilute nutrient solution and at the same time or later the plant tops were removed near the base with a sharp razor blade. As the exudate appeared on the cut stump, it was removed completely by touching the origin point of a paper chromatogram to the stump. Thus for a given plant, the exudate could be transferred quantitatively, making it simple to carry out time-course studies. The first collections after cutting off the plant top utilized all the exudate that appeared. When collection times were further apart than ½ hour, there was often an excess of it which dripped from the stump or which was removed by blotting with a piece of filter paper. For these later collections the stump was blotted with filter paper ½ hour before the final sampling and all the exudate appearing after that was used for the chromatographic analysis.

Chromatograms were run on unwashed Whatman #1 paper. They were developed in the first direction with water-saturated phenol and in the second direction with butanol-propionic acid-water (1). After development, the chromatograms were exposed to no-screen x-ray film to establish the exact location of the P^{32}. These spots were cut into small pieces for counting with an end-window Geiger-Müller tube.

RESULTS AND DISCUSSION

On chromatograms of the exudate collected from the stump of decapitated barley seedlings, tomato, sunflower, bean, and willow there were 3 radioactive phosphorus spots which had R_f characteristics given in table I. The discovery of only 3 phosphorus-containing compounds in the exudate was surprising, since the phosphorus was neither all inorganic phosphate nor was there the multiplicity of known organophosphorus compounds associated with respiration which would be found in whole tissue. The two phosphorus-containing compounds besides inorganic phos-

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