Effect of Light and Dark on the Intracellular Fate of Photosynthetic Products[1, 2]

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In attempts to distinguish the various pools of metabolic products that may be chemically or physically separated in photosynthesizing cells, C^{14} labeled compounds have been fed to cells in a number of kinetic studies (2, 3, 7, 10). From these studies it is evident that the photosynthetic intermediates, presumably within the chloroplasts, rapidly acquire the same specific activity as the substrate CO_2 and that C^{14} enters other (nonplastid) pools more slowly, presumably in the hyaloplasm or vacuoles. Smith, Bassham, and Kirk (10) showed that the active pool of alanine becomes saturated with C^{14} within 30 minutes of photosynthesis by *Chlorella* in $C^{14}O_2$. In contrast, the specific activity curve of glutamic acid passes through an inflection after 20 minutes and continues to increase slowly. This was interpreted as evidence for a secondary slowly labeled pool (presumably outside of the plastid). The active pools of several amino acids were found to represent 20 to 25 % of the total pools.

It has become possible with the development of the nonaqueous method of isolating chloroplasts to study the intracellular distribution and movement of some of the early products of photosynthesis (4, 13). Leaching of water soluble components during chloroplast isolation in buffered sucrose or saline solutions renders the current aqueous methods of little use in such studies. It has been shown that there is a rapid movement of certain carbon compounds from the chloroplast into its cytoplasmic environment while other carbon compounds particularly those directly concerned with the carbon cycle of photosynthesis (4) are unable to pass readily out of the plastid.

It should be possible by using the nonaqueous method of isolating chloroplasts to determine the actual sizes of the chloroplast pools of various compounds and to compare these pools with those found outside of the plastids. The present study involved the use of the nonaqueous method of isolation of plastids to follow the influence of light and dark on the fate of some of the early products of photosynthesis.

Materials and Methods

Two-month-old greenhouse grown tobacco plants (*Nicotiana rustica* L.) were placed in the dark at 25° for 48 hours and then brought into the light for 1 hour. Well expanded leaves were cut from the plants and their petioles immediately placed in one-fourth strength Hoagland nutrient solution. A small portion of the petiole was cut off under the solution.

The excised leaves were placed in a plexiglass feeding chamber and illuminated for 5 minutes prior to and during the $C^{14}O_2$ feeding by a double bank of Gro-lux lights providing an intensity of about 1500 ft-c at each leaf surface. CO_2 was generated in a closed feeding chamber from $BaCO_3$ containing from 100 to 500 μc C^{14} depending on the experiment. The gas was continuously circulated over the leaves. The volume of the system containing 2 leaves was approximately 2500 ml and the initial concentration of CO_2 was about 0.08 %. After the $C^{14}O_2$ feeding, the leaves were either transferred to $C^{14}O_2$ free air for the light and dark studies or they were plunged immediately into liquid nitrogen. In the latter case the time necessary to remove the leaf and immerse it completely in the liquid nitrogen was 4 to 6 seconds. Frozen leaves were transferred to lyophilization flasks at —18° and freeze dried at that temperature. The dried leaves were stored over P_2O_5 at —18° in a vacuum desiccator or used directly.

Chloroplasts were isolated nonaqueously by Stocking's method (11) with the modification that a density gradient was used. Plastids isolated in aqueous media were obtained by cutting the leaf into cooled 0.5 M sucrose and grinding in a Waring blendor for 3, 10 second bursts. The suspension was filtered through 2 thicknesses of cheese cloth and the chloroplasts separated by a 7-minute centrifugation at 1000 *g* following a 1-minute centrifugation at 200 *g*. The total time for isolation was 25 minutes.

The chloroplasts and heavy pellets were extracted with 80 % (v/v) ethanol and twice with water at 77 and 85°. The fractionation of the extracts into amino acids, organic acids and phosphorylation compounds was accomplished by adsorption of the amino acids on Amberlite IR-120 cation exchange resin

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and adsorption of the organic acids and phosphorylation compounds on Dowex 1×8 anion exchange resin. Sugars were present in the neutral fraction not adsorbed on the resins. Amino acids were eluted from the Amberlite IR-120 resin with 10 % NH_4OH. Organic acids and phosphorylated compounds were eluted together from the Dowex 1×8 resin by 1.5 M $(NH_4)_2CO_3$. Two dimensional paper chromatographic separation of the C^{14} labeled compounds was carried out on oxalic acid washed Whatmann No. 4 paper with phenol-water and butanol-water-propionic acid solvent systems (1). Kodak Medical X-ray film (Royal Blue) was used for the location of the radioactive spots.

The amount of radioactivity in the homogenates was determined by plating aliquots on nickel planchets and counting them with a Tracerlab gas-flow counter. The amount of radioactivity in the individual spots on the paper chromatograms was determined by cutting out the portion of the filter paper containing the spot, placing it in a vial with scintillation fluid, and counting the radioactivity in a Packard-Tri-Carb liquid scintillation spectrophotometer.

Hydrolysis of starch in the alcohol-water insoluble pellets was done enzymatically. The insoluble pellets were homogenized in about 25 ml of distilled water and heated at 90° in a water bath for 3 hours to gelatinize the starch. The samples were cooled to room temperature and incubated for 30 minutes with a solution of malt amylase (Merck). The hydrolysis was repeated with amylase and then the samples were centrifuged at 15,000 g for 30 minutes, and the radioactivity in the hydrolysate and the amylase insoluble fraction was determined. The hydrolysate was evaporated to dryness, taken up in a small volume of water, and chromatographed immediately. Glucose and maltose were identified as the products of the hydrolysis.

Protein of the insoluble pellets was hydrolyzed in 10 ml of 6 N HCl in sealed glass tubes for 22 hours at 105°. The amino acids in the hydrolysate were adsorbed on Amberlite IR-120, eluted with 10 % NH_4OH, separated by paper chromatography, and identified by radioautography or by ninhydrin spraying.

When the insoluble pellet was hydrolyzed in 6 N HCl before amylase hydrolysis, the bulk of the radioactivity appeared in 1 spot which was identified as levulinic acid. Similar hydrolysis of purified, C^{14}-starch gave the same radioactive spot.

Results

Intracellular Distribution of the Early Products of Photosynthesis. After 10 minutes in $C^{14}O_2$, 10.5 % of the total fixed radioactivity was found in chloroplasts isolated in 0.5 M sucrose, and 10 % of this radioactivity was removed by 1 washing in the same medium (table I). Approximately 80 % of the C^{14} that did remain in the chloroplast after aqueous isolation was found to be present as water and alcohol insoluble compounds such as starch. Thus, only about 3 % of the C^{14} labeled water and alcohol soluble compounds remained in the plastids after aqueous isolation. In contrast to the low radioactivity of the aqueously isolated chloroplasts, nonaqueously isolated plastids contained as much as 54 % of the total C^{14} after 10 minutes feeding in $C^{14}O_2$. These results together with the observation that less than 1 % of the radioactivity was present in the solvent indicate that the nonaqueous method prevented significant leaching of the early products of photosynthesis from the plastids during their isolation by this method. In the following experiments, chloroplasts were isolated by the nonaqueous method.

Table I. *Distribution of C^{14} in Fractions Isolated From Leaves Treated with $C^{14}O_2$ for 10 Minutes in the Light*

Fraction	Cpm × 10^4 per 1 mg leaf chlorophyll
0.5 M Sucrose isolation*	
200 g 1 min precipitate	
cellular debris, nuclei, cell walls	3.5
1000 g 7 min precipitate	
unwashed chloroplasts	9.8
1000 g 7 min supernatant	
nonplastid cytoplasmic and vacuolar material	116.8
Once washed chloroplasts	8.0
Wash solution	2.2
Nonaqueous isolation**	cpm × 10^4 per 50 mg dry leaf
Chloroplast fraction	273.7
Nonaqueous fraction	231.4
Solvent (Hexane + CCl_4)	1.95

* Leaf fed CO_2 from 4.0 mg $BaC^{14}O_3$ (specific activity 23.1 μc/mg).

** Two leaves fed CO_2 from 6.0 mg $BaC^{14}O_3$ (specific activity 61.1 μc/mg) in the same chamber.

The effects of light and dark on the fate of compounds labeled with C^{14} after 10 minutes photosynthesis in $C^{14}O_2$ was to be studied, consequently the intracellular distribution of some of the individual compounds that were labeled with C^{14} was determined immediately after the 10 minutes of photosynthesis. It is evident (table II) that during the 10 minutes nearly half of the C^{14} moved out of the plastids. As one would expect, the chloroplasts are the primary sites of the C^{14} label in the insoluble compounds since starch is the major insoluble compound labeled at this time though smaller amounts of protein are also labeled. The other groups of labeled compounds located predominantly in the chloroplasts are the early-labeled phosphorylated compounds about 80 % of which are still in the plastids after 10 minutes. This is in agreement with Heber and Willenbrink's re-

Table II. *Intracellular Distribution of C^{14} Labeled Compounds after 10 Minutes Photosynthesis in $C^{14}O_2$*

Fraction	Cpm × 10^4 per 50 mg dry wt leaf	
	Chloroplast fraction	Nonchloroplast fraction
Total C^{14} fixed	229.0	201.0
Alcohol and water soluble compounds	216.8	199.7
Amino acids	30.4	43.1
Sugars	124.4	123.8
Phosphorylated compounds	24.9	7.8
Citric acid	0.34	0.68
Malic acid	3.18	4.34
Glyceric acid	19.3	15.3
Insoluble compounds	23.9	4.5

* Two tobacco leaves fed CO_2 from 6.0 mg of $BaC^{14}O_3$ (Specific activity 61.6 μc/mg).
** Chloroplasts isolated nonaqueously.

sults (4) on the plastid location of the phosphorylated intermediates in the carbon cycle.

In contrast to the location of the phosphorylated compounds, C^{14} labeled citric acid is found predominantly outside of the chloroplast while C^{14} malic acid is more nearly equally distributed between the plastid and cytoplasm, and C^{14} glyceric acid is somewhat more concentrated in the plastid at this time.

The Effect of Light and Dark on the Early C^{14} Labeled Compounds of Photosynthesis. After 10 minutes photosynthesis in $C^{14}O_2$, an additional 30 minutes in air in the dark had little effect on the distribution of the C^{14} among the 4 groups: 1) alcohol and water insoluble compounds, 2) amino acids, 3) sugars, and 4) phosphorylated compounds and organic acids (fig 1). A slight drop in the sugars and a corresponding increase in the insoluble compounds was observed. In marked contrast to this is the effect of 30 minutes of light. In the light, the label in the amino acid fraction decreased sharply while a corresponding increase in the insoluble fraction was observed. The C^{14} in the fraction containing phosphorylated compounds and organic acids also decreased in the light. This is chiefly a result of a decrease in C^{14} in the phosphorylated compounds in the chloroplasts as will be shown later.

In order to determine how much of the radioactivity found in the alcohol and water insoluble fraction was due to starch, the insoluble pellet was incubated with amylase. After 10 minutes photosynthesis with $C^{14}O_2$, the tobacco leaf had 6.2 % of its total radioactivity in starch (table III). This value did not increase when the leaves were allowed to stay in the dark for 30 additional minutes. However, when the leaf was kept in the light in air but in the absence of $C^{14}O_2$ for the same period of time, C^{14} moved into the starch fraction so that the starch accounted for over 20 % of the total radioactivity in

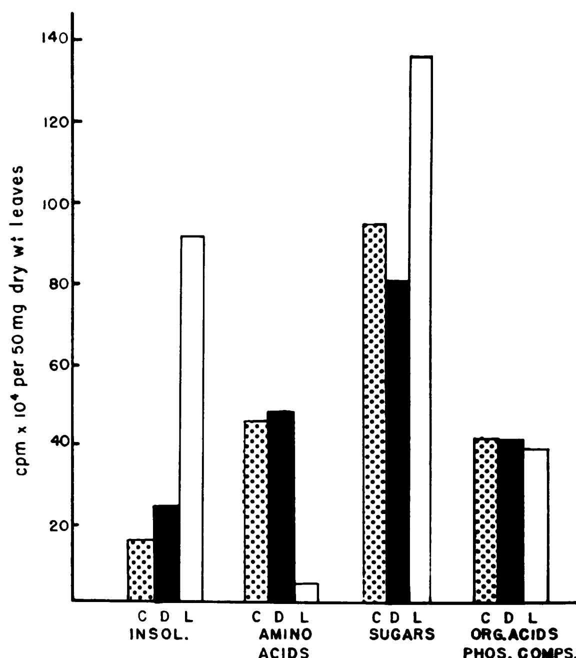

Fig. 1. Effect of light and dark on the distribution of radioactivity in different compounds of tobacco leaves. C, control, C^{14} distribution after 10 minutes photosynthesis in $C^{14}O_2$; D, C^{14} distribution after 30 minutes dark in air after 10 minutes photosynthesis in $C^{14}O_2$; L, C^{14} distribution after 30 minutes light in air after 10 minutes photosynthesis in $C^{14}O_2$. In each case 6.0 mg $BaC^{14}O_3$ (specific activity 25 μc/mg) were used.

the leaf. Some radioactivity in the insoluble fraction, less than 5 % of the total radioactivity, was insoluble in amylase and upon acid hydrolysis atter amylase digestion yielded amino acids and is considered to be protein.

Table III. *Effect of Light and Dark on the Radioactivity in Starch in Leaves Fed $C^{14}O_2$*

Fraction	Cpm × 10^4 per 50 mg dry wt leaf*		
	10 min photosynthesis in $C^{14}O_2$	10 min photosynthesis in $C^{14}O_2$ + 30 min dark in air	10 min photosynthesis in $C^{14}O_2$ + 30 min light in air
Total activity	229.2	203.1	237.7
Alcohol and water soluble comps	208.1	182.0	175.0
Total alcohol and water insoluble	21.1	21.1	62.7
Amylase soluble material (starch)	14.2	10.7	52.4
Amylase insoluble	6.9	10.4	10.3

* In each case approximately 5.6 mg $BaC^{14}O_3$ (specific activity 27 μ/mg) were used.

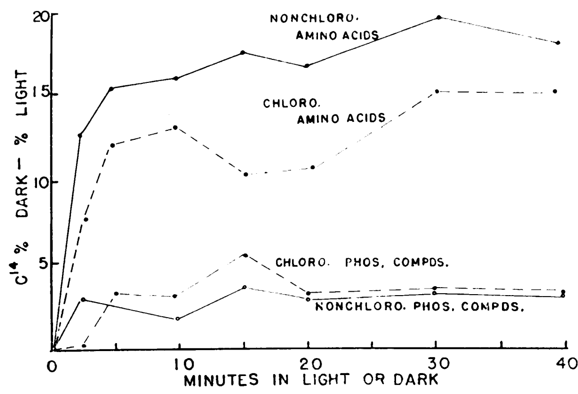

FIG. 2. Effect of light and dark on the C^{14} in the amino acid and phosphorylated compounds of chloroplast and nonchloroplast fractions of tobacco leaves. The data are plotted as differences between the C^{14} content (expressed as percent of the total C^{14} in the plastid or nonplastid fractions) in a particular compound from dark treated leaves less the C^{14} content in the compound from light treated leaves. This method of expressing these results emphasizes the actual differences that occur as a result of the light and dark treatments and minimizes other variations.

Time Sequence Studies. In order to determine the effect of light on the rate of change of C^{14} distribution among the several types of compounds, paired leaves, after 10 minutes photosynthesis in $C^{14}O_2$, were sampled at various times from 2 to 40 minutes after being in $C^{14}O_2$ free air either in the light or in the dark.

At the end of 10 minutes photosynthesis, somewhat more than 50 % of the C^{14} labeled amino acids were located outside of the chloroplasts (table II). At this time the amino acids represented 14.6 % of the C^{14} of the chloroplast and 22.3 % of the C^{14} of the nonchloroplast material. After an additional 2 minutes in the light in the absence of $C^{14}O_2$, the amino acids in the chloroplast dropped to 8.9 % of the total C^{14} of the chloroplasts, but after 2 minutes in the dark they represented 16 % of the total plastid C^{14}. At the same time the amino acids outside of the chloroplast accounted for 11.4 % of the nonplastid C^{14} in the light and 23.4 % in the dark or a difference of 12 %. The rapid establishment of a difference in the C^{14} content in the amino acids between the light and dark treatments is shown in figure 2. Within 10 to 15 minutes this difference reached its highest value and was slightly greater for the nonchloroplast amino acids than for the chloroplast amino acids.

In contrast to the effect of light in decreasing the C^{14} level in the amino acid pools, the changes that occurred in the radioactivities of the insoluble fraction and in sugars under light and dark treatments were the reverse (fig 3). The additional radioactivity in the alcohol and water insoluble fraction in the light treated leaves was almost completely located in the chloroplasts while that in the sugars was somewhat more pronounced outside of the chloroplasts. Like the amino acids the phosphorylated compounds showed a more rapid turnover in the light than in the dark. A look at the effects of light and dark on the turnover in the pools of some

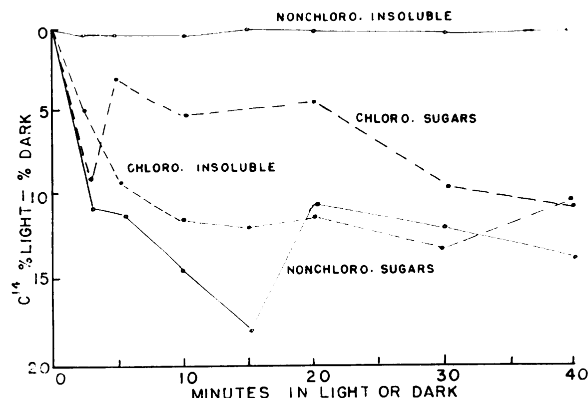

FIG. 3. Effect of light and dark on the C^{14} in the sugar and insoluble fractions of chloroplast and nonchloroplast components of tobacco leaves. All leaves photosynthesized for 10 minutes in $C^{14}O_2$ before they were placed in air either in the light or in the dark. Since the light increased the C^{14} content in these compounds, the data are plotted as differences between the C^{14} in a particular compound from light treated leaves less the C^{14} content of the compound from dark treated leaves.

of the individual compounds is instructive (table IV). When $C^{14}O_2$ is replaced by $C^{12}O_2$ in the light, phosphorylated compounds and sugar in the chloroplasts lost C^{14}. Glycine, serine, and glyceric acid both inside and outside of the plastids all showed a decrease in their C^{14} content in the light. Only in the case of serine was light necessary for this change. Serine actually increased in C^{14} in the dark.

A continued increase in the label in asparagine, aspartic acid, glutamic acid, and malic acid occurred in both the light and the dark when $C^{14}O_2$ was removed. On the other hand alanine changed very little in the light but increased in C^{14} content in the dark.

Discussion

Knowledge of the intracellular localization of compounds is necessary for a complete understanding of leaf metabolism. To obtain information on the intracellular localization of compounds, one must separate the cell parts from each other so that the isolated units neither loose nor gain solutes during the isolation. Isolating the chloroplasts in an aqueous medium causes severe leaching of water soluble mate-

Table IV. *Light and Dark Effects on the Distribution of C^{14} between the Chloroplast and the Rest of the Cell*

<table>
<tr><th rowspan="3">Substance
Time in light or dark after 10 min photosynthesis in $C^{14}O_2$</th><th colspan="6">Percent of total cellular radioactivity located in individual compounds found in nonaqueously isolated plastids and in the nonchloroplast cell material obtained from leaves treated either in the light or in the dark</th></tr>
<tr><th colspan="2">Chloroplast fraction</th><th colspan="2">Nonchloroplast fraction</th><th colspan="2">Effects of</th></tr>
<tr><th>Leaf in light</th><th>Leaf in dark</th><th>Leaf in light</th><th>Leaf in dark</th><th>Light</th><th>Dark</th></tr>
<tr><td>Aspartic acid</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.76</td><td colspan="2">0.99</td><td></td><td></td></tr>
<tr><td>20–40 min*</td><td>0.90</td><td>2.23</td><td>2.03</td><td>4.77</td><td>Increase**</td><td>Increase**</td></tr>
<tr><td>Glutamic acid</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.04</td><td colspan="2">0.04</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.83</td><td>0.64</td><td>1.09</td><td>0.72</td><td>Increase</td><td>Increase</td></tr>
<tr><td>Glycine</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">3.35</td><td colspan="2">3.96</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.29</td><td>0.42</td><td>0.44</td><td>0.64</td><td>Decrease</td><td>Decrease</td></tr>
<tr><td>Serine</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">3.35</td><td colspan="2">3.04</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.68</td><td>6.21</td><td>1.02</td><td>7.49</td><td>Decrease</td><td>Increase</td></tr>
<tr><td>Alanine</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.55</td><td colspan="2">0.57</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.38</td><td>1.94</td><td>0.57</td><td>1.99</td><td>None</td><td>Increase</td></tr>
<tr><td>Asparagine</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.19</td><td colspan="2">0.09</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>1.34</td><td>0.89</td><td>2.67</td><td>1.10</td><td>Increase</td><td>Increase</td></tr>
<tr><td>Citric acid</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.08</td><td colspan="2">0.16</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.42</td><td>0.75</td><td>1.50</td><td>1.17</td><td>Increase**</td><td>Increase**</td></tr>
<tr><td>Glyceric acid</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">4.49</td><td colspan="2">3.56</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>0.04</td><td>0.12</td><td>0.07</td><td>0.28</td><td>Decrease</td><td>Decrease</td></tr>
<tr><td>Malic Acid</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">0.74</td><td colspan="2">1.01</td><td></td><td></td></tr>
<tr><td>20–40 min</td><td>2.81</td><td>2.68</td><td>9.67</td><td>6.00</td><td>Increase**</td><td>Increase**</td></tr>
<tr><td>Sugars</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">33.0</td><td colspan="2">25.5</td><td>Decrease</td><td>Decrease</td></tr>
<tr><td>20–40 min</td><td>23.2</td><td>25.4</td><td>33.5</td><td>23.7</td><td colspan="2">in plastids only</td></tr>
<tr><td>Phosphorylated compounds</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0 min</td><td colspan="2">5.78</td><td colspan="2">1.82</td><td>Decrease</td><td>Increase</td></tr>
<tr><td>20–40 min</td><td>1.55</td><td>5.32</td><td>1.56</td><td>3.77</td><td>in plastids</td><td>outside of plastids</td></tr>
</table>

* Average of 3 determinations made at 20, 30, and 40 minutes after $C^{14}O_2$ removed.
** Greatest effect outside of the plastids.

rials from them. Isolation of the chloroplasts in organic solvents eliminates the leaching effect of the aqueous media (table I). An important question regarding the validity of the nonaqueous technique is whether or not chloroplasts isolated in this way are contaminated by vacuolar or cytoplasmic material. The absence of major vacuolar contamination in chloroplasts isolated by the nonaqueous technique has been demonstrated (11), and electron and light microscope observations (12) failed to reveal significant cytoplasmic contamination. Since less than 1 % of the total radioactivity appeared in the solvent, it was concluded that water soluble radioactive compounds do not leach readily into the solvent during this isolation procedure.

The intimate relationship between the chloroplasts and their cytoplasmic environment is indicated by the rapid exchange of materials between them (table II).

After 10 minutes of photosynthesis almost half of the fixed carbon had moved into the cytoplasm. It should be kept in mind that the sizes of the pools of the various compounds in and outside of the chloroplasts cannot be estimated from these data since the specific activities were not determined. It also should be remembered that the volume relationships between chloroplasts, cytoplasm, and vacuoles are not known and that there are many cells in the complex leaf tissues that do not have chloroplasts. Although the actual concentration of a given compound at a cell locus cannot be determined from these data, a comparison of the differences in distribution of the labeled compounds between chloroplasts and the rest of the cell yields information on intracellular movements of the compounds and is important in reference to the subsequent metabolism of these compounds.

If as Heber and Willenbrink indicate (4), a considerable part of the carbon taken up in photosynthesis is translocated from the chloroplasts into the cytoplasm in the form of phosphorylated compounds, there must be a rapid release of the phosphorous when these compounds reach the cytoplasm since a major portion of the phosphorylated compounds still are present in the chloroplasts after 10 minutes of photosynthesis (table IV). One must also visualize a rapid translocation of phosphorous from the cytoplasm into the chloroplast to compensate for the phosphorous lost during the outward transport of the phosphates. However, it has been reported that inorganic phosphate is transferred only slowly across the chloroplast envelope (9). A possible alternative hypothesis to the transport of inorganic phosphate across the chloroplast envelope would be that phosphatases located in the envelope function in the transfer of phosphorylated sugars in such a way that the free sugar would be liberated into the cytoplasm and inorganic phosphorous released into the plastid where it could be recycled in the phosphorylation processes. Evaluation of such a hypothesis must await more detailed information on the intracellular location of phosphatases.

Chloroplasts contain only small pools of asparagine, aspartic acid, glutamic acid, citric acid, and malic acid. This is indicated by the relatively low values for C^{14} present in the chloroplast pools of these compounds after 10 minutes of photosynthesis and the fact that these pools saturate relatively rapidly (7, 9). The nonplastid pools of these compounds continue to become labeled slowly at the expense of labeled precursors over a longer period of time (table IV).

The effects of light and dark on the distribution of radioactivity in different compounds in leaves previously fed $C^{14}O_2$ (fig 1) indicate that carbon of the amino acids is moved in the light into carbohydrates, in particular starch. This effect of light is similar to Ibrahim's observation (5) that leaf discs of 5 species of angiosperms were able to incorporate C^{14} from uniformly labeled L-tryosine-C^{14} into sugars and organic acids in the light but that there was virtually no sugar or organic acid synthesis from L-tryosine in the dark. Similarly, Tolbert's group (6, 8) demonstrated that serine-3-C^{14} was converted by wheat leaves in the light to sucrose and not converted in the dark. Glycolate was metabolized in the light to phosphate esters and sucrose, but in the dark the C^{14} from glycolate moved only into glycine, serine, and glycerate and not into phosphate esters or sucrose. Wang and Waygood (15) found that sugar synthesis from glycine was stimulated by the light. A rapid loss of C^{14} from a compound, when the $C^{14}O_2$ around a leaf in the light is replaced by $C^{12}O_2$, would point to a significant turnover in the pool of this particular compound in the light with the carbon moving in a normal fashion from this compound into compounds that are labeled subsequently. Phosphorylated compounds and sugars in the chloroplasts but not outside of the chloroplasts, glycine, glyceric acid, and serine both inside and outside of the chloroplasts all showed this trend (table IV). The turnover of carbon in these compounds is directly dependent on light energy only in the cases of the phosphorylated compounds in the plastid and of serine. In the dark, serine actually increased in C^{14} probably as a result of the movement of label from glycine into serine and the absence of serine metabolism in the dark.

Since light had little effect on the total label in the phosphorylated compounds in the hyaloplasm but did have an effect on these compounds in the chloroplasts, one would expect that the plastid envelope presents a barrier to the rapid movement of the more stable phosphorylated compounds out of the cytoplasm. In contrast, little difference was observed between the fate of serine inside and outside of the plastid. This would indicate that serine readily passes across the plastid membrane.

The existence of a glycolate pathway from the early carbon products of photosynthesis to sucrose has been proposed for wheat leaves (6, 7, 14, 15). The light and dark experiments reported here could be explained by the operation of such a cycle with a light dependent (ATP and NADPH dependent) sequence located between serine and carbohydrates:

$$\text{glycine} \rightarrow \text{serine} \xrightarrow{\text{light}} \text{carbohydrate.}$$

The carbohydrate that appears to be the final product of this series of reactions in tobacco leaves is starch.

Summary

The usefulness of the nonaqueous method of isolating chloroplasts has been verified for a study of the intracellular fate of the early products of photosynthesis and a study of the pools of various metabolites located inside and outside of chloroplasts.

The existence of very rapidly labeled pools of glycine, serine, alanine, and glycolic acid as well as

phosphorylated compounds in the chloroplasts of tobacco has been demonstrated. Serine appears to move freely through the chloroplast membrane while the membrane appears to be less permeable to certain phosphorylated compounds.

Light induces a rapid turnover in the amino acid pools with carbon moving from them into carbohydrates, particularly starch. This effect results chiefly from the rapid metabolism of serine in the light but not in the dark. The results presented are in agreement with the suggestion that the glycolate pathway from early carbon products of photosynthesis to carbohydrates is operative in tobacco leaves.

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