Glycolate, Glycerine, Serine, and Glycerate Formation during Photosynthesis by Tobacco Leaves

J. L. Hess and N. E. Tolbert

From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823

SUMMARY

Formation of 3-phosphoglycerate, glycolate, glycerate, and serine by tobacco leaves was analyzed between 4 and 60 sec of $^{14}C_2$ fixation during photosynthesis. With decreasing time, the percentage of the total $^{14}C$ incorporated into 3-phosphoglycerate increased; the percentage into the other compounds, including glycolate, decreased to nearly zero. At 4 or 11 sec 3-phosphoglycerate and glycerate were predominantly carboxyl labeled; glycolate was uniformly labeled. At 4 sec the specific activity of the carboxyl group of 3-phosphoglycerate was about 100-fold greater than that of either carbon atom of glycolate. Carbon atoms 2 and 3 of 3-phosphoglycerate were uniformly labeled at 4 or 11 sec and had a 10-fold higher specific activity than the carbon atoms of glycolate. 3-Phosphoglycerate became uniformly labeled between 30 and 60 sec, and the specific activities of carbon atoms of 3-phosphoglycerate and glycolate became similar. Addition of $\alpha$-hydroxy-2-pyridinemethanesulfonate did not alter the initial rates of labeling or specific activity of 3-phosphoglycerate or glycolate, but glycolate-$^{14}C$ accumulated while the percentage of $^{14}C$ decreased in products formed from glycolate, such as glycine, serine, glycerate, and sucrose.

Glycine, like glycolate, was uniformly labeled even at the shortest times. After 4 sec serine was equally labeled in carbon atoms 1 and 2. Carbon atom 3 was slightly less active. In 11 sec serine was uniformly labeled. Results were consistent with serine formation from glycolate and glycine and a small unlabeled C1 pool. After 4 sec in the presence of the glycolate oxidase inhibitor, the small amount of labeled serine was slightly carboxyl labeled, as if part of the serine came from 3-phosphoglycerate.

In 60 sec, tobacco leaves incorporated 12% of the total $^{14}C$ in glycerate. The percentage distribution of $^{14}C$ in glycerate carbon atoms was similar to that in 3-phosphoglycerate. The specific activity of glycerate carbon atoms was one-one hundredth that of 3-phosphoglycerate at 4 and 11 sec and one-tenth that of 3-phosphoglycerate at 60 sec.

The glycolate pathway in green plants describes the metabolic sequence from phosphoglycolate and glycolate through glyoxy-

* This investigation was supported by National Science Foundation Grant GB 4154 and approved for publication as Journal Article No. 3841 of the Michigan Agricultural Experiment Station.

The source of glycolate formation during $CO_2$ fixation by leaves of higher plants remains unknown, although over 50% of the total fixed carbon may be passing through this metabolite (1, 2). The kinetics of $^{14}C$ accumulation in uniformly labeled glycolate by algae and higher plants has indicated that glycolate was formed as a metabolic product from one or more components of the photosynthetic carbon cycle (3-5). Similar conclusions have been made from metabolism studies with possible $^{14}C$-labeled precursors (see review by Tolbert (1)). Nevertheless, in recent years three independent investigations have concluded that glycolate biosynthesis may result from an alternate $CO_2$ fixation pathway in photosynthesis. From electron spin resonance signals on manganese-deficient Chlorella, Tanner et al. (6) suggested that glycolate was a product formed directly from the condensation of 2 carbon dioxide molecules. Warburg and Krippahl (7) observed that Chlorella, during 1 hour of photosynthesis, converted 92% of the fixed $CO_2$ into glycolate. These results, however, are probably in error owing to nonphotosynthetic metabolism of the algal carbohydrates to glycolate. Zelitch (8) has reported that, after $CO_2$ fixation by tobacco leaves for 2 or 5 min, the glycolate was uniformly labeled and the specific activity of these carbon atoms was greater than the specific activity of the carboxyl carbon atom of phosphoglycerate. Therefore, he suggested that glycolate must originate from a previously undetected $CO_2$-fixation pathway.

In all of Zelitch's experiments $\alpha$-hydroxy-2-pyridinemethanesulfonate was used as an inhibitor of glycolate oxidation (9) in order to accumulate sufficient glycolate for degradation and specific activity determinations. In this report we present data from tobacco leaves, in both the absence and presence of this inhibitor, on the rate of labeling of glycolate and the specific activity of the glycolate carbon atoms formed during $CO_2$ photosynthesis from 4 to 60 sec. Similar data are presented for 3-phosphoglycerate, glycine, serine, and glycerate formation. The large amount of glycolate formation and metabolism in leaves during photosynthesis is emphasized. The results substantiate the concept that glycolate is formed from components of the photosynthetic carbon cycle and permit an analysis of the significance of the glycolate pathway.

EXPERIMENTAL PROCEDURE

Photosynthesis Experiments—Young leaves about 30 to 40 cm long from field grown tobacco (Nicotiana tabacum) were cut from plants at 9 a.m. and immediately set in a beaker containing

1 N. E. Tolbert and H. Holzer, unpublished results.
either water or 0.01 M \( \alpha \)-hydroxy-2-pyridinemethanesulfonate at pH 3.5. The leaves were removed at 5-min intervals so that each leaf remained in the solution 1 hour before it was used.

The leaves took up enough solution to become turgid in 10 min, since partial chlade and aeration insured high transpiration rates. After 1 hour, the bottom part of the leaf was cut off so that the remaining tip was about 15 cm long and 10 cm across at the widest point. It was immediately put into a photosynthetic chamber and exposed to \(^{14}\)CO\(_2\). After extraction of the methanol-water-soluble components, the dry weights of these leaf tips varied between 384 and 471 mg.

The plexiglas photosynthetic chamber of 375-ml volume (11 x 15 x 1.7 cm) had a removable cover and was connected to a vacuum pump. Before the experiment was begun, about 200 \( \mu \)C of \(^{14}\)CO\(_2\) were released inside a 60-ml separatory funnel by addition of lactic acid through a syringe cap on top of the funnel to a known amount of Ba\(^{14}\)CO\(_3\) (52 \( \mu \)C per mg). The separatory funnel with the \(^{14}\)CO\(_2\) was connected to the chamber, and a partial vacuum of 75 to 90 mm of mercury was quickly pulled on the chamber and the vacuum line closed. Then, at zero time, the stopcock of the funnel containing the \(^{14}\)CO\(_2\) was opened and nearly simultaneously a second operator removed the syringe cap so that the \(^{14}\)CO\(_2\) was swept into the chamber. These conditions gave a final CO\(_2\) concentration in the chamber between 0.2 and 0.3\%. The leaf was held perpendicular to the sunlight of an intensity of 7000 footcandles as measured with a Weston photometer. At the end of the experimental period the lid of the chamber was slid off while another operator poured the methanol-water-diethylamine, 80: 10:20:1 (11). The \( ^{14} \)C-glycerate fractions from the Dowex 1 column were combined, evaporated to approximately 0.5 ml, and hydrolyzed with alkaline phosphatase at pH 7.5 at 30\(^\circ\)C for 2 hours. The liberated glycerate was then further purified by the paper chromatography system just described.

Quantitative colorimetric assays were used for the determination of glycolate (12) and glycinate (13). The specific activity as counts per min per \( \mu \)mole of carbon was obtained as the product of the specific activity in counts per min per \( \mu \)mole of acid and the percentage of the \(^{14}\)C in a particular carbon atom of the acid as determined by degradation.

Determination of Specific Activities—Since large samples of a compound were needed for degradation and specific activity determinations, about one third of each sample was evaporated to 10 ml and passed through a Dowex 1 (acetate) column (1.3 x 7.5 cm) in order to separate P-glycerate from glycolate and glycinate (8). The fractions containing the organic acids were evaporated under reduced pressure at 35\(^\circ\)C and further purified by paper chromatography in a basic solvent system (butanol-95\% ethanol-water-diethylamine, 50:10:20:1) (11). The P-glycerate fractions from the Dowex 1 column were combined, evaporated to approximately 0.5 ml, and hydrolyzed with alkaline phosphatase at pH 7.5 at 30\(^\circ\)C for 2 hours. The liberated glycerate was then further purified by the paper chromatography system just described.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery</th>
<th>(^{14})C recovered</th>
<th>(^{14})C added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformly labeled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-P-glycerate-(^{14})C</td>
<td>96</td>
<td>33.9</td>
<td>33.3</td>
</tr>
<tr>
<td>Serine-(^{14})C</td>
<td>97</td>
<td>95.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Serine-3-(^{14})C</td>
<td>96</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Glycine-1-(^{14})C</td>
<td>100</td>
<td>87.5</td>
<td>12.4</td>
</tr>
<tr>
<td>Glycine-2-(^{14})C</td>
<td>96</td>
<td>1.3</td>
<td>98.7</td>
</tr>
</tbody>
</table>
| Glycolate-1-\(^{14}\)C   | 100      | 99.5                 | 0.5             | 99.5            | 0.5
| Glycolate-2-\(^{14}\)C   | 90       | 14.0                 | 86.0            | 12.6            | 87.4 |
| Uniformly labeled      |          |                      |                 |
| glycolate-\(^{14}\)C     | 95       | 54.5                 | 45.5            | 51.4            | 48.6 |

* These values were consistent for degradations of different time periods, and, therefore, were not attributed to randomization during the degradation.
which were isolated by us from long term photosynthesis experiments. The percentage recovery was calculated by counting an aliquot of nondegraded material and comparing it with the total $^{14}$C recovered. For P-glycerate, glycerate, serine, and glycine the percentage distribution of $^{14}$C was calculated from the sum of the amounts of $^{14}$C recovered at each degradation step. The total yield of $^{14}$C from glycolate-$^{2-14}$C was consistently low and varied between 90 and 95%. The recovery of the first carbon atom of glycolate was excellent (99%), but complete recovery of the second carbon atom was not achieved. Thus, uniformly labeled glycolate yielded a 55:45 ratio for carbon atom 1 to carbon atom 2 if the distribution was based on total $^{14}$C recovered in the degradation. This ratio became 51:49 if the distribution in carbon atom 1 was based on the total $^{14}$C added in the degradation and carbon atom 2 was calculated by difference. Thus in the results, although the complete degradation was run each time as a check, the $^{14}$C distribution in glycolate was calculated upon the percentage of $^{14}$C recovered in the first carbon atom with respect to the total added to the reaction.

RESULTS

Rate of Formation—A nearly linear rate of $^{14}$CO$_2$ fixation during the first 60 sec in the experiment without $\alpha$-hydroxy-2-pyridine-methanesulfonate (Fig. 1) indicated that CO$_2$ availability did not become severely rate limiting. The percentage distribution of $^{14}$C fixed into the various products of photosynthesis from 4 to 60 sec is shown in Fig. 2. The percentage of the total $^{14}$CO$_2$ fixed in P-glycerate decreased with time from about 30% at 4 sec to 10% at 60 sec while the $^{14}$C fixed into glycolate, glycerate, serine, and sucrose increased with time. These are data similar to those originally obtained by Benson and Calvin (5) with algae and other plants and by Orth, Tolbert, and Jiminez (3) with algae.

**Fig. 1.** Rate of total $^{14}$CO$_2$ fixation by tobacco leaves which were treated 1 hour before the isotope experiment with either water (O---O) or 0.01 M $\alpha$-hydroxy-2-pyridine-methanesulfonate (●—●).

**Fig. 2.** Percentage distribution of total $^{14}$C in P-glycerate, glycolate, glycine, and serine, glycerate, and sucrose which were formed during photosynthesis from 4 to 300 sec. Control (O---O), 0.01 M $\alpha$-hydroxy-2-pyridine-methanesulfonate (●—●).
Such data have been interpreted as indicating that initially 3-P-glycerate contained most of the newly fixed 14C and that other compounds including glycolate are formed subsequently.

An estimation of the amount of 14C in the products of CO2 fixation is obtained from the product of total fixation and the percentage of the 14C in each (Fig. 3). In the presence of a-hydroxy-2-pyridinemethanesulfonate, 18% of the total soluble 14C was found in glycerate after 30 sec of 14CO2 fixation, while in experiments lasting 2 to 5 min as much as 50% of the 14C accumulated in glycolate. Evidently the inhibition by a-hydroxy-2-pyridinemethanesulfonate of glycolate oxidase, as first described by Zelitch (2), caused a block in the movement of 14C through the glycolate pathway. Thus, subsequent incorporation of 14C into glycine, serine, glycerate, and sucrose appeared to be blocked by the a-hydroxy-2-pyridinemethanesulfonate as shown in Figs. 2 and 3. An approximately reciprocal relationship existed between the accumulation of glycolate-14C in the presence of the inhibitor and the decrease of 14C in the subsequent products of the glycolate pathway.

**Specific Activities** In Table II are presented the specific activities of the chromatographically pure samples of 3-P-glycerate, glycerate, and glycolate from the various 14CO2-fixation experiments. The percentage distributions of the 14C among the carbon atoms of these compounds are shown in Table III. From these data the specific activity of each carbon atom of 3-P-glycerate, glycerate, and glycolate have been calculated (Table IV). These values represent the average of at least two analyses of each radioactive product, the results of which varied less than 10%.

The results in Tables II, III, and IV are from single experiments with approximately the same size of leaf and amount of 14CO2. In all of the experiments the specific activities and the percentage distribution of 14C in the compounds were not affected by the presence of a-hydroxy-2-pyridinemethanesulfonate, even though this compound greatly altered the pool size or the total amount of 14C which accumulated in these compounds.

For the shortest experiment (4 sec) the specific activity of 3-P-glycerate had already reached a high value (Table IV), but the compound was still predominantly carboxyl labeled. Between 30 and 60 sec the 3-P-glycerate became uniformly labeled (Table III). Glycerate had at all times equal distribution of the label in both carbon atoms. The percentage distribution of 14C in free glycerate was similar in all cases to that in 3-P-glycerate. However, the specific activity of glycerate was initially 100-fold less than that of 3-P-glycerate, and by 60 sec the specific activity of glycerate was still approximately one-tenth that of 3-P-glycerate. Such differences could be attributed to a large free glycerate reservoir and a small 3-P-glycerate pool.

We can also compare carbon atoms 2 and 3 of 3-P-glycerate with the carbon atoms of glycolate (Table IV). After 4 sec of photosynthesis, the specific activity of carbon atoms 2 or 3 of 3-P-glycerate was the same and about 10 times greater than the specific activity of the glycolate carbon atoms. By 11 sec, however, glycolate carbon atoms and carbon atoms 2 and 3 of 3-P-glycerate had nearly the same specific activity.

**Glycine and Serine**—Glycine was uniformly labeled (Table III) as had been previously reported (16). Distribution of 14C label in serine was of particular interest in order to differentiate between the movement of carbon during photosynthesis from uniformly labeled glycolate through glycine to serine and the formation of serine from carboxyl-labeled 3-P-glycerate. In the 4- and 11-sec control experiments without the sulfonate inhibitor, the serine was essentially uniformly labeled in carbon atoms 1 and 2, even though the 3-P-glycerate and glycate were predominantly carboxyl labeled. Such data have been cited to support the concept of very rapid glycine and serine formation by the glycolate...
TABLE III
Percentage distribution of 14C in compounds isolated from tobacco leaves after photosynthesis in 14CO2

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Pretreatment</th>
<th>3-Phosphoglycerate</th>
<th>Glycolate</th>
<th>Glycerate</th>
<th>Glycine</th>
<th>Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Water</td>
<td>83.9</td>
<td>7.9</td>
<td>2.3</td>
<td>61.1</td>
<td>28.9</td>
</tr>
<tr>
<td>4</td>
<td>α-Hydroxy-2-pyridine- methanesulfonate</td>
<td>87.4</td>
<td>7.1</td>
<td>2.5</td>
<td>63.7</td>
<td>24.3</td>
</tr>
<tr>
<td>11</td>
<td>Water</td>
<td>64.9</td>
<td>19.1</td>
<td>7.9</td>
<td>32.1</td>
<td>40.1</td>
</tr>
<tr>
<td>11</td>
<td>α-Hydroxy-2-pyridine- methanesulfonate</td>
<td>64.0</td>
<td>16.5</td>
<td>19.5</td>
<td>30.1</td>
<td>48.1</td>
</tr>
<tr>
<td>30</td>
<td>Water</td>
<td>51.1</td>
<td>23.0</td>
<td>25.9</td>
<td>24.1</td>
<td>34.9</td>
</tr>
<tr>
<td>30</td>
<td>α-Hydroxy-2-pyridine- methanesulfonate</td>
<td>49.6</td>
<td>28.1</td>
<td>22.3</td>
<td>31.4</td>
<td>48.6</td>
</tr>
<tr>
<td>60</td>
<td>Water</td>
<td>36.7</td>
<td>33.3</td>
<td>30.1</td>
<td>26.4</td>
<td>35.9</td>
</tr>
<tr>
<td>60</td>
<td>α-Hydroxy-2-pyridine- methanesulfonate</td>
<td>43.1</td>
<td>28.8</td>
<td>28.1</td>
<td>49.9</td>
<td>51.1</td>
</tr>
</tbody>
</table>

- Extrapolated to zero time was increasing, while the percentage in glycolate extended to zero (Fig. 2). At 4 sec the carboxyl group of 3-P-glycerate had 100-fold higher specific activity than either carbon atom of glycolate (Table IV). At this time the 3-P-glycerate was predominantly carboxyl labeled, but glycolate was uniformly labeled (Table III). Carbon atoms 2 and 3 of 3-P-glycerate were equally labeled at 4 sec, and their specific activity was 10-fold greater than the glycolate carbon atoms. By about 30 sec 3-P-glycerate was uniformly labeled, and the specific activities of all the carbon atoms of both 3-P-glycerate and glycolate were nearly equal. Thus the rapidity of 14C labeling of 3-P-glycerate as well as the glycolate reservoir by tobacco leaves is emphasized. However, the possibilities that glycolate might be labeled before 3-P-glycerate or by a separate CO2-fixation process were not indicated by our data. Since the specific activity of glycolate was initially much less than the carboxyl group or even carbon atoms 2 and 3 of 3-P-glycerate, labeled glycolate cannot be the precursor of 3-P-glycerate. The reverse, that 3-P-glycerate could be the precursor of glycolate, was possible. Until the enzymes for glycolate biosynthesis are known, pathway rather than from 3-P-glycerate (16). In leaves treated with α-hydroxy-2-pyridinemethanesulfonate and then exposed to 14CO2 for 4 sec, the serine was carboxyl-labeled, but less carboxyl-labeled than the 3-P-glycerate or glycerate. Apparently, in leaves treated with α-hydroxy-2-pyridinemethanesulfonate, serine synthesis from 3-P-glycerate occurred, but as glycolate began to accumulate, the incomplete inhibition of the glycolate pathway permitted limited synthesis of uniformly labeled serine. Thus, two pathways for serine formation may be detected in plants, but the serine becomes uniformly labeled more rapidly than 3-P-glycerate.

TABLE IV
Specific activities of carbon atoms in compounds isolated from tobacco leaves after photosynthesis in 14CO2

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Pretreatment</th>
<th>3-Phosphoglycerate</th>
<th>Glycolate</th>
<th>Glycerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Water</td>
<td>57.6 × 10^3cpm/mole carbon</td>
<td>0.66</td>
<td>0.70</td>
</tr>
<tr>
<td>4</td>
<td>α-Hydroxy-2-pyridine-methanesulfonate</td>
<td>26.6 × 10^3cpm/mole carbon</td>
<td>0.20</td>
<td>0.36</td>
</tr>
<tr>
<td>11</td>
<td>Water</td>
<td>12.6 × 10^3cpm/mole carbon</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>11</td>
<td>α-Hydroxy-2-pyridine-methanesulfonate</td>
<td>13.6 × 10^3cpm/mole carbon</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>30</td>
<td>Water</td>
<td>7.7 × 10^3cpm/mole carbon</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>30</td>
<td>α-Hydroxy-2-pyridine-methanesulfonate</td>
<td>5.5 × 10^3cpm/mole carbon</td>
<td>6.8</td>
<td>7.7</td>
</tr>
<tr>
<td>60</td>
<td>Water</td>
<td>29.6 × 10^3cpm/mole carbon</td>
<td>8.4</td>
<td>7.7</td>
</tr>
<tr>
<td>60</td>
<td>α-Hydroxy-2-pyridine-methanesulfonate</td>
<td>36.6 × 10^3cpm/mole carbon</td>
<td>16</td>
<td>17</td>
</tr>
</tbody>
</table>

- Rate of Labeling of Glycolate and 3-P-Glycerate—The possibility of forming uniformly labeled glycolate from 2 14CO2 or 14C-1 molecules motivated this investigation. By both the criteria of rate of 14C labeling and the specific activity of each carbon atom, glycolate was labeled after 3-P-glycerate by tobacco leaves during 14CO2 fixation. The rate of labeling of both compounds is rapid; however, the plot of the percentage of 14C in 3-P-glycerate extrapolated to zero time was increasing, while the percentage in glycolate extended to zero (Fig. 2). At 4 sec the carboxyl group of 3-P-glycerate had 100 fold higher specific activity than other carbon atom of glycolate (Table IV). At this time the 3-P-glycerate was predominantly carboxyl labeled, but glycolate was uniformly labeled (Table III). Carbon atoms 2 and 3 of 3-P-glycerate were equally labeled at 4 sec, and their specific activity was 10-fold greater than the glycolate carbon atoms. By about 30 sec 3-P-glycerate was uniformly labeled, and the specific activities of the carbon atoms of both 3-P-glycerate and glycolate were nearly equal. Thus the rapidity of 14C labeling of 3-P-glycerate as well as the glycolate reservoir by tobacco leaves is emphasized. However, the possibilities that glycolate might be labeled before 3-P-glycerate or by a separate CO2-fixation process were not indicated by our data. Since the specific activity of glycolate was initially much less than the carboxyl group or even carbon atoms 2 and 3 of 3-P-glycerate, labeled glycolate cannot be the precursor of 3-P-glycerate. The reverse, that 3-P-glycerate could be the precursor of glycolate, was possible. Until the enzymes for glycolate biosynthesis are known,
the possibility will remain that CO₂ fixation for glycolate formation differs from that for 3-P-glycerate formation. Labile intermediates between CO₂ and glycolate would distort the interpretation of the rate of labeling, and large pool sizes of these labile intermediates and of glycolate would lower the observed specific activity.

The use of α-hydroxy-2-pyridinemethanesulfonate as an inhibitor of glycolate oxidase caused an immense accumulation of glycolate-¹⁴C during photosynthesis. However, this inhibitor did not alter, at all, the initial rate of labeling or the specific activity of 3-P-glycerate or glycogulate.

The conclusions in this manuscript differ from those reported for photosynthesis by tobacco leaf discs by Zelitch (8), who found that the carbon atoms of glycolate had a higher specific activity than the carboxyl group of 3-P-glycerate. Several experimental differences between these two reports may have accounted for the different results. Although Zelitch used only leaves treated with α-hydroxy-2-pyridinemethanesulfonate in order to accumulate more glycolate-¹⁴C, we found no large alteration in the specific activity of glycolate in the presence of this inhibitor. In our experiments, the pretreatment of leaves with the inhibitor for 1 hour before addition of ¹⁴CO₂ could have caused an increase in the unlabeled pool of glycolate, thus reducing its specific activity. However, since the isolate experiments were performed at both a higher light intensity and CO₂ concentration than during the pretreatment, the observed similarity of specific activities between the treated and untreated leaves might be expected.

Zelitch used experimental ¹⁴CO₂-fixation periods of 2 and 5 min, and then during the termination of his experiments, 30 sec in air were required to move the leaf tissue from the Warburg vessel to the killing solution. In our experiments killing was accomplished in about 2 sec. Our results indicate that after 30 sec of ¹⁴CO₂ fixation both 3-P-glycerate and glycolate had attained equal specific activity. In a killing procedure of nearly 30 sec, ¹³CO₂ from the air would have replaced a significant amount of the ¹⁴C from the carboxyl group of 3-P-glycerate.

Serine and Glycolate Pathway in Vivo—To evaluate the significance of the glycolate pathway in vivo, Zelitch has used hydroxymethanesulfonates as inhibitors of glycolate oxidase (9). Recently, however, these sulfonate compounds have been shown to affect several other processes (17, 18); thus the accumulation of glycolate in the presence of these inhibitors may be the expression of other factors. Nevertheless, with the inhibitors, the significance of the glycolate pathway may be indicated not only by the accumulation of glycolate, but also by alteration of ¹⁴C in subsequent products from glycolate. In the presence of the α-hydroxy-2-pyridinemethanesulfonate we observed a large decrease in the amount of ¹⁴C in glycine, serine, glycerate, and sucrose which approximated the ¹⁴C accumulation in glycogulate-¹⁴C.

Glycine and serine occupy a unique metabolic position in plants with respect to their rate of ¹⁴C labeling during photosynthesis (5, 16). In very short periods of time they are labeled uniformly with ¹⁴C and in much larger amounts than other amino acids except aspartate and glutamate which are only initially carboxyl labeled from CO₂ exchange reactions. There are two pathways for serine formation in plants. One is by the glycogulate pathway and forms uniformly labeled serine (16). The other pathway which also occurs in microorganisms and animals, is from 3-P-glycerate and initially during photosynthesis would lead to carboxyl-labeled serine. The latter pathway has been shown to function in chloroplasts (14) and in algae where the glycolate pathway is blocked by the absence of glycolate oxidase.²

In the present control experiments, the formation of large amounts of uniformly labeled glycine and serine in 4 and 11 sec when 3-P-glycerate was still carboxyl labeled substantiates our prior publication (16) on the rapidity and magnitude of serine formation. In the presence of α-hydroxy-2-pyridinemethanesulfonate, which inhibits glycolate oxidase, glycine and serine were labeled at about 50% of the rate observed in the absence of the inhibitor. At 4 sec the serine was slightly carboxyl labeled, and thus could have come from 3-P-glycerate. It appears that if serine formation from glycolate is blocked by α-hydroxy-2-pyridinemethanesulfonate, then an alternate pathway from 3-P-glycerate continues the rapid serine synthesis. The reason is unknown for serine formation by one of the pathways in larger amounts than other amino acids, but the function of specific amino acids in membrane transport is a possibility, e.g. aspartate (19) and serine (20). That the glycine was, at all times, uniformly labeled, probably indicates an incomplete inhibition of glycogulate oxidation in the presence of α-hydroxy-2-pyridinemethanesulfonate.

Glycerate—A substantial amount of the total ¹⁴C activity was accumulated in glycogulate during ¹⁴CO₂ photosynthesis by leaves of tobacco (Fig. 3) as has been observed in sugar beet, bean, wheat, and tomato plants. If, as Benson et al. (21) suggested, the rapid appearance of labeled glycogulate was an artifact due to phosophatase action on the 3-P-glycerate during the killing procedure, the 3-P-glycerate-¹⁴C to glycogulate-¹⁴C ratio should be nearly constant. As indicated in Fig. 3, this ratio was about 5 for the 4-sec experiment when only a small amount of glycogulate had been labeled, but the ratio decreased to less than 1 since by 00 sec there was more ¹⁴C in glycogulate than 3-P-glycerate. The glycogulate, however, had a similar percentage distribution of ¹⁴C among its carbon atoms as did 3-P-glycerate (Table III). These results imply that the glycogulate came from a hydrolysis in vivo of the 3-P-glycerate, as has been suggested by Mortimer (22), and not from hydrolysis during the killing procedure.

Another source of glycogulate besides 3-P-glycerate would be from the glycogulate pathway (1). When specifically labeled glycogulate, glycine, or serine were fed to leaves, they were all rapidly converted in the light to glycogulate and sucrose; the labeling patterns were consistent with the direct formation of glycogulate from serine (16, 23, 24). In the present experiments glycogulate, glycine, and serine were nearly uniformly labeled by ¹⁴CO₂ in the shortest periods of time. Thus, if part of the glycogulate were formed by the glycogulate pathway, glycogulate ought to have been more uniformly labeled than 3-P-glycolate. However, the percentage distribution of ¹⁴C in glycogulate was similar to that in 3-P-glycolate and not to that in serine; apparently the bulk of the glycogulate was produced from 3-P-glycolate. At short times, uniformly labeled glycogulate from the glycogulate pathway was either not formed, or formed at such a metabolically active site that it did not accumulate in sufficient quantities to alter the percentage distribution from that obtained from carboxyl-labeled 3-P-glycolate.

Free glycogulate might be a storage component. As such, its initial specific activity would be much less than the specific activity of 3-P-glycolate. Similarly the free glycogulate reservoir should be less metabolically active than 3-P-glycolate of the

² J. L. Hess and N. E. Tolbert, unpublished data.
photosynthetic carbon cycle. However, the distribution of $^{14}$C in glycerate was equilibrated as rapidly as that in 3-P-glycerate (Table III), even though the specific activity of glycerate remained one-tenth that of 3-P-glycerate (Table IV). Other recent reports (25, 26) indicate that the P-glycerate reservoirs in the chloroplast and cytoplasm may be in much more rapid equilibrium than are other compounds of the photosynthetic carbon cycle.

Acknowledgment—We are grateful to Karen Swanson for skilled technical assistance during part of this work.

REFERENCES
Glycolate, Glycine, Serine, and Glycerate Formation during Photosynthesis by Tobacco Leaves
J. L. Hess and N. E. Tolbert


Access the most updated version of this article at http://www.jbc.org/content/241/23/5705

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/241/23/5705.full.html#ref-list-1