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# Comparison of the Effectiveness of Glycolic Acid and Glycine as Substrates for Photorespiration

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## ABSTRACT

**Considerable evidence exists that the carboxyl-carbon atom of glycolic acid is the primary source of the $CO_2$ produced during photorespiration by leaves of many species of plants, including tobacco. Experiments were conducted to determine whether glyoxylate or glycine, both products of glycolic acid metabolism, is the more immediate precursor of photorespiratory $CO_2$.**

**Illuminated tobacco leaf disks were floated on 18 mM solutions of glycolate-1-$^{14}$C or glycine-1-$^{14}$C in $CO_2$-free air. The $^{14}CO_2$ released and the $^{14}$C content of several postulated intermediates were determined when the substrate solutions were provided alone or with one of the following: 9 mM α-hydroxy-2-pyridinemethanesulfonic acid, an inhibitor of the oxidation of glycolate to glyoxylate; 9 mM isonicotinyl hydrazide, an inhibitor of the conversion of glycine to serine; or 18 mM nonradioactive glycine or glycolate with the other radioactive substrate.**

**Both inhibitors decreased the rate of photorespiration in tobacco leaf disks by the $^{14}$C-assay. The α-hydroxy-2-pyridinemethanesulfonic acid severely blocked $^{14}CO_2$ production and labeling of the glycolate pathway from glycolate-1-$^{14}$C. Isonicotinyl hydrazide had little effect on the $^{14}CO_2$ released from glycine-1-$^{14}$C although the glycine to serine conversion was severely inhibited.**

**These results and other data in the literature indicate that the glycolate pathway of carbohydrate metabolism does not supply sufficient $CO_2$ during the synthesis of serine from glycine to account for the rates of photorespiration observed in many species. A direct decarboxylation of glyoxylate is more likely the main source of photorespiratory $CO_2$.**

Ample evidence exists that the large quantities of $CO_2$ produced in many photosynthetic tissues in the light arise primarily from the carboxyl-carbon atom of glycolic acid, an early product of photosynthesis (1, 2). This evidence comes from experiments showing parallel effects on glycolate metabolism and photorespiration brought about by changes in the oxygen and $CO_2$ concentrations in the ambient atmosphere, the use of biochemical inhibitors and $^{14}$C-labeled metabolites on intact tissues, and assays of enzyme activities in leaf extracts and in isolated leaf organelles (4, 5, 23). However, there is still uncertainty whether most of the photorespiratory $CO_2$ arises directly from decarboxylation of glyoxylate produced in the glycolate oxidase reaction or during the conversion of glycine to serine at a later step in the glycolate pathway of carbohydrate synthesis shown schematically in Figure 1 (ref. 23, pp. 204–208).

I previously suggested that the $CO_2$ in photorespiration could result from the well known nonenzymic oxidation of glyoxylate by $H_2O_2$ produced in the glycolate oxidase reaction in the absence of catalase (21). Tolbert and his co-workers (13) later showed convincingly that glycolate oxidase is mostly found in leaf peroxisomes, and that these cytoplasmic organelles contain a great excess of catalase activity that would decompose any $H_2O_2$ produced there. Hence, it seemed unlikely that the decarboxylation of glyoxylate could occur by such a mechanism in the peroxisomes, although it might occur elsewhere in the cell. They suggested instead (6, 7) that the glycolate pathway (11, 15) must first produce glycine (Fig. 1), and that the $CO_2$ is released during the complex hydroxymethyltransferase condensation of two glycine molecules to produce serine. Therefore, according to this view, glycine rather than glyoxylate would be the more immediate precursor of the $CO_2$ arising during photorespiration.

The rate of $CO_2$ production during photorespiration must be at least 50 μmoles $CO_2$/mg chlorophyll·hr (ref. 23, p. 200), and earlier attempts to demonstrate an enzymic decarboxylation of glyoxylate produced such low rates that its importance in photorespiration was discounted (6, 7). However, I have recently shown that spinach chloroplasts supplemented with $Mn^{2+}$ ions decarboxylate glyoxylate-1-$^{14}$C rapidly enough to account for known photorespiratory rates by an enzymic photooxidation reaction that does not involve glycine (24). Hence an adequate possible mechanism for the direct decarboxylation of glyoxylate is now known.

On the other hand, for several reasons the glycolate pathway seems inadequate in its present formulation to account for rates of photorespiration indicated above. Enzyme activity assays for the $CO_2$-producing reaction in this pathway are too slow (9, 12), and the enzymic activity of the essential transamination of glyoxylate to glycine is also far from rapid enough to account for photorespiration by this pathway (12). Moreover, the glycolate pathway (Fig. 1) requires that for every four molecules of glycolate oxidized to produce ultimately one molecule of glucose, no more than two molecules of $CO_2$ (or 25% of the glycolate-carbon) may be lost by this mechanism. Since rates of $CO_2$ production during photorespiration often exceed those of net photosynthesis (ref. 23, p. 200), the stoichiometry of this pathway as written could not release sufficient $CO_2$ to account for known rates of photorespiration.

Other workers have shown that glycolate-1-$^{14}$C and glycine-1-$^{14}$C produced $^{14}CO_2$ at about equal rates in the light in pea leaves based on the percentage of the total $^{14}$C metabolized (8), while in tobacco leaf segments it appeared that glycine-1-$^{14}$C is a somewhat better precursor of $^{14}CO_2$ when expressed on the basis of the $^{14}$C absorbed by the tissues (7). The formation of $^{14}CO_2$ and other $^{14}$C-intermediates from glycine-1-$^{14}$C has also

been examined in the high photorespiration species *Atriplex hastata,* and the rate of labeling of serine-$^{14}C$ was about the same as in species with low rates of photorespiration (9).

In this paper the observations of these investigators have now been extended in experiments designed to test whether glycine is a mandatory intermediate in photorespiration and whether the direct decarboxylation of glyoxylate may occur in intact tissues. The release of $^{14}CO_2$ and the $^{14}C$-labeling pattern of various intermediates of the glycolate pathway have been examined in tobacco leaf tissue supplied with glycolate-1-$^{14}C$ or glycine-1-$^{14}C$ at the same pH under conditions known to produce high rates of photorespiration. In these experiments, a study was made of the effect of α-HPMS,[1] an inhibitor of glycolate oxidase (3, 18, 20, 22) and INH, a known inhibitor of the glycolate pathway (3, 10). The addition of carrier glycolate and glycine to the radioactive substrate was also investigated for their effect on $^{14}CO_2$ production and the labeling of intermediates during photorespiration. The results show that glycine is not a mandatory precursor of the large quantities of $CO_2$ arising during photorespiration.

## MATERIALS AND METHODS

**$^{14}C$-Photorespiration Assay.** Details and limitations of this method have been previously described (22). Six tobacco leaf disks (*Nicotiana tabacum,* var. Havana Seed) 1.6 cm in diameter with a fresh weight of about 240 mg (0.36 mg chlorophyll) were tied together with a thread and floated on water in large (75 ml) Warburg flasks in the light in air at 30 C for a preliminary period. The system was closed and $^{14}CO_2$ was released into the atmosphere in such an amount that it was assimilated by the tissue in about 15 min. After at least 45 min, at zero time, $CO_2$-free moistened air was rapidly swept through the system and the released $^{14}CO_2$ was trapped in 1 M ethanolamine during a period of illuminance and subsequently during darkness. The $^{14}CO_2$ content was determined by scintillation counting, and the results were expressed as the ratio of the $^{14}CO_2$ evolved in the light to that in the dark.

When a substrate or inhibitor was added, after the preliminary period in light as described above, at zero time, the water in the flasks was quickly replaced with the new solution and $CO_2$-free air was swept through the system as before. Stomatal widths were determined from silicone rubber impressions (19) of the lower epidermis of leaf disks.

**Substrates and Inhibitors.** Sodium glycolate-1-$^{14}C$ (Amersham-Searle) solution was passed through a column of Dowex-50 $H^+$ cation exchange resin. Carrier glycolic acid was added to the effluent to make the final concentration 40 mM, and it was 50% neutralized with potassium bicarbonate to give a solution at pH 3.8. Glycine-1-$^{14}C$ (New England Nuclear) was diluted with carrier glycine to make the final concentration 40 mM and used without further treatment. The α-HPMS was obtained as "2-pyridylhydroxymethanesulfonic acid" from Aldrich Chemical Company and was recrystallized from 25% ethyl alcohol before use. This compound is now available from Columbia Organic Chemicals, Columbia, South Carolina or from Fluka AG, Buchs, Switzerland. A 10 mM solution has a pH of 3.8 when 25% neutralized with potassium bicarbonate. INH was purchased from Eastman Kodak Company. Sodium tartrate buffer was used because tartrate is not metabolized by tobacco tissue (14).

**Separation of Compounds for $^{14}C$-Determination and Degradation.** At the end of experiments in which leaf disks were supplied with glycolate-1-$^{14}C$, or glycine-1-$^{14}C$, the leaf disks tied together with the thread were quickly removed from the flasks, plunged into 200 ml of water to remove adsorbed radioactive substrate, and then immediately into boiling 20% ethyl alcohol. After 3 min, the killed tissue was ground in a Ten-Broeck homogenizer, and the suspension was made up to 25 ml. The total $^{14}C$ was determined in a small sample, and thus the total $^{14}C$ metabolized could be calculated by subtracting the substrate-$^{14}C$ remaining in the tissue from the total $^{14}C$. Since the specific radioactivity of the substrates (cpm per nmole) was known, the $^{14}C$ content in various compounds or fractions could be expressed as nmoles of $^{14}C$ present.

The suspension was centrifuged, and the residue was washed twice by centrifugation with water. The combined supernatant fluids were placed on a column of Dowex-1 acetate anion exchange resin (17). The neutral and basic compounds were first eluted with water. Elution was then continued with 4 M acetic acid. A glycolic acid fraction, containing glycolic and glyceric acids, and the glyoxylic acid were separated and collected by this method.

The glycolic acid fraction was concentrated by blowing a stream of air upon the surface while the solution was maintained at 45 C, and the glycolic and glyceric acids were completely separated from each other by descending paper chromatography in an alkaline solvent system as earlier described (20). These compounds were eluted from the paper, and the $^{14}C$ was determined as before.

The neutral and basic fraction was placed on a column of Dowex-50 $H^+$ and the neutral compounds (carbohydrates) were eluted with water and collected. The amino acids were then eluted with 2.0 N $NH_4OH$. A complete separation of the glycine and serine was obtained in this fraction by high voltage electrophoresis on Whatman No. 3 MM paper (3000 v or 67 v $cm^{-1}$, 20 min) in a solution of formic and acetic acids (20 ml concentrated formic acid and 80 ml concentrated acetic acid to 1 liter) at pH 1.9. The glycine and serine were located on adjacent areas that were detected with ninhydrin, and these amino acids were separately eluted from the paper for the determination of their $^{14}C$ content. When a known quantity of glycine-$^{14}C$ was mixed with serine and taken through the above procedure, 97% of the $^{14}C$ was found in the glycine area of the paper and 3% in the serine; 85% of the $^{14}C$ supplied was recovered as glycine-$^{14}C$.

Glycolate was degraded (20) by oxidizing it to glyoxylate with glycolate oxidase in the presence of excess catalase at pH 9.0, followed by an oxidative decarboxylation with ceric sulfate to $CO_2$ (derived from the C-1) and formate (obtained from the C-2) of glycolate. Recoveries within 1 to 2% of the total were obtained by this method with known mixtures of glycolate-1-$^{14}C$ and -2-$^{14}C$.

## RESULTS

**Effect of α-HPMS and INH on Photorespiration.** α-HPMS at 10 mM is known to inhibit the $^{14}CO_2$ released during photorespiration without affecting dark respiration in the $^{14}C$-assay (22), even when the stomata are not closed by the α-HPMS (at 35 C). This inhibitor also decreased the specific radioactivity of the $^{14}CO_2$ released in the light about 30%, while it had no effect on the specific radioactivity of released $^{14}CO_2$ in darkness (3). The transaminase and serine hydroxymethyltransferase inhibitor (10) INH at a concentration of 10 mM also lowered the specific radioactivity of photorespiratory $^{14}CO_2$ (3). Table I shows that both inhibitors greatly diminished the ratio of $^{14}CO_2$ released in the light compared with the dark in the standard assay when leaf disks were floated at 30 C on 9 mM solutions at pH 3.8. At this temperature, part of the inhibitory effect of α-HPMS was undoubtedly caused by

[1] Abbreviations: α-HPMS: α-hydroxy-2-pyridinemethanesulfonic acid; INH: isonicotinyl hydrazide.

stomatal closure (19), while INH inhibited photorespiration without decreasing stomatal width even at 30 C.

**Comparison of Glycolate-1-$^{14}$C and Glycine-1-$^{14}$C as Substrates for Photorespiration.** The release of $^{14}CO_2$ in light from tobacco leaf disks floated on 20 mM solutions of glycolate-1-$^{14}$C increases greatly at 35 C compared with 25 C when based on the total $^{14}$C metabolized (22). No such increase occurs when glycolate-2-$^{14}$C is substituted (22) or when formate-$^{14}$C is the substrate (Fig. 1). Figure 2 appears to show that the rate of $^{14}CO_2$ released from glycine-1-$^{14}$C is somewhat faster than from glycolate-1-$^{14}$C at 35 C. However, when the $^{14}CO_2$ produced at the end of 45 min was expressed on the basis of the total $^{14}$C metabolized, which tends to correct for differences in the uptake of the substrates, the decarboxylation rates were the same.

The experiment in Table II was carried out at 30 C. Leaf disks were floated on solutions containing radioactive substrate and inhibitor or nonradioactive carrier as indicated. The addition of 9 mM $\alpha$-HPMS inhibited the release of $^{14}CO_2$ from glycolate-1-$^{14}$C greatly, and less from glycine-1-$^{14}$C, but much of the apparent inhibition of glycine decarboxylation was probably caused by stomatal closure (Table I). The $\alpha$-HPMS also greatly decreased the labeling of intermediates of the glycolate pathway from glycolate-1-$^{14}$C, especially glycine and serine as well as carbohydrates. However, this inhibitor did not block serine formation from glycine-1-$^{14}$C and decreased labeling of carbohydrate much less than it did from glycolate-1-$^{14}$C.

The addition of INH (Table II) to the system did not inhibit the decarboxylation of glycolate or glycine or the formation of radioactive products of the glycolate pathway from added labeled glycolate, although INH severely inhibited the formation of serine-$^{14}$C from glycine-1-$^{14}$C. Labeling of carbohydrate from glycine-1-$^{14}$C was also unaffected by INH, thus indicating that the glycine-serine conversion is not essential for the production of $CO_2$ during photorespiration or for the synthesis of carbohydrate from glycine.

Supplying nonradioactive glycine together with glycolate-1-$^{14}$C to increase the size of the nonradioactive pool did not decrease the rate of $^{14}CO_2$ released or the labeling in carbohydrates (Table II). The addition of carrier glycolate to glycine-1-$^{14}$C did not greatly diminish the decarboxylation of glycine or the formation of radioactive carbohydrate, although the labeling in serine was decreased.

The last column in Table II shows that the total $^{14}$C metabolized by each labeled substrate was about the same in the various treatments except for glycolate-1-$^{14}$C in the presence of $\alpha$-HPMS. Thus none of the treatments, with this one exception, presumably affected the rate of uptake or the overall utilization of the substrate supplied. If one pathway was blocked, the substrate must have been utilized by another sequence of reactions.

A degradation of the glycolate-$^{14}$C remaining in the tissue (although not necessarily in the cells) was carried out in all instances where glycolate-1-$^{14}$C was provided or where glycine-1-$^{14}$C was supplied in the presence of $\alpha$-HPMS. With glycolate-1-$^{14}$C added by itself, 96% of the $^{14}$C was in C-1; with $\alpha$-HPMS, 99% was in C-1; with INH, 99% was in C-1; and with nonradioactive glycine, 98% was in C-1. In contrast, where gly-

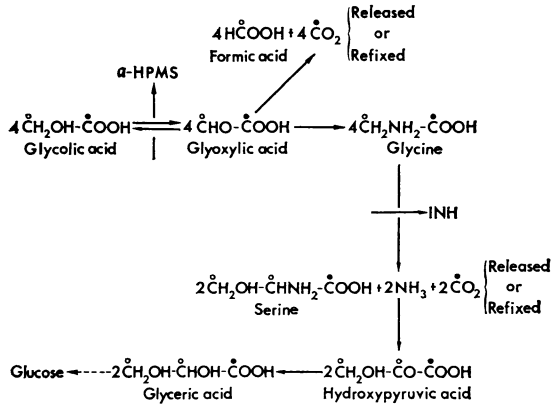


FIG. 1. Schematic diagram of the glycolate pathway of carbohydrate synthesis showing likely sources of photorespiratory $CO_2$. ●: from C-1 of glycolic acid; ○: from C-2 of glycolic acid.

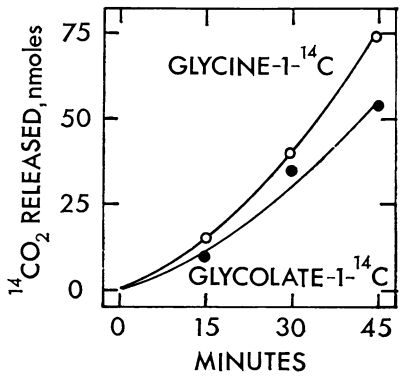


FIG. 2. Rate of $^{14}CO_2$ released from tobacco leaf disks floated on solutions of glycolate-1-$^{14}$C or glycine-1-$^{14}$C at 35 C. Six disks were floated on 1.5 ml of water in large Warburg flasks (75 ml volume) in light (1500 ft-c) in air at 35 C for 30 min. At zero time, the water was removed and replaced with either 40 $\mu$moles of potassium glycolate-1-$^{14}$C ($4.16 \times 10^6$ cpm) at pH 5.0 or 40 $\mu$moles of glycine-1-$^{14}$C ($10.4 \times 10^6$ cpm) in a final volume of 2.0 ml. $CO_2$-free air was then passed through the system at 250 ml/min and trapped in 60 ml of 1 M ethanolamine. The $^{14}CO_2$ content was measured at the times shown. At the end of the experiment the disks were killed and the total $^{14}$C metabolized was determined as described in "Materials and Methods." The $^{14}CO_2$ production in this experiment was identical with both substrates when the results were expressed on the basis of the total $^{14}$C metabolized.

Table I. *Effect of $\alpha$-Hydroxy-2-pyridinemethanesulfonic Acid and Isonicotinyl Hydrazide on the Photorespiration and Dark Respiration of Tobacco Leaf Disks in the $^{14}$C-Assay*

Leaf disks were floated in water in large Warburg flasks which were shaken at 30 C under 900 ft-c of illuminance. After 15 min in air, the system was closed and 5 $\mu$moles of $^{14}CO_2$ ($2.06 \times 10^6$ cpm) were released into the closed system. After 45 min of $^{14}CO_2$ fixation, the water was removed and quickly replaced with 2.0 ml of 10 mM sodium tartrate buffer at pH 3.8, or buffer containing either 9 mM $\alpha$-HPMS (pH 3.8) or 9 mM INH. At zero time, $CO_2$-free air was passed rapidly through the flasks and the $^{14}CO_2$ released between 5 and 35 min in light and subsequently between 15 and 45 min in darkness was collected and measured (22).

| Flask Contents | $^{14}CO_2$ Released in Light | $^{14}CO_2$ Released in Dark | $^{14}CO_2$ in Light/Dark | Mean Stomatal Width[1] |
|---|---|---|---|---|
| | *cpm* | | *ratio* | $\mu$ |
| Buffer | 315,000 | 61,800 | 5.1 | 7.1 |
| + $\alpha$-HPMS | 119,000 | 42,500 | 2.8 | 2.9 |
| + INH | 83,800 | 32,600 | 2.6 | 7.5 |

[1] Determined on leaf disks treated similarly in a companion experiment after "completion" of the entire simulated light period. Before buffer was added, at "zero time," the mean stomatal width was 7.1 $\mu$.

Table II. *Release of $^{14}CO_2$ and Distribution of $^{14}C$ in Tobacco Leaf Disks Supplied Glycolate-1-$^{14}C$ or Glycine-1-$^{14}C$ in the Light in $CO_2$-Free Air*

The leaf disks were maintained in water in large Warburg flasks for 1 hr in the light (1500 ft-c) at 30 C. At zero time the water was replaced with 2.0 ml of 10 mM sodium tartrate buffer at pH 3.8 containing 18 mM potassium glycolate-1-$^{14}C$ (pH 3.8, $5.95 \times 10^6$ cpm) or 18 mM glycine-1-$^{14}C$ ($13.1 \times 10^6$ cpm). Inhibitors, when present, were 9 mM, and carrier glycine or glycolate were added where shown at a final concentration of 18 mM. After 30 min in a rapid stream of $CO_2$-free air, the experiment was terminated and $^{14}C$ determinations were made as described in "Materials and Methods."

| Flask Contents | $^{14}C$ Content | | | | | | | $^{14}C$ in Glycolate Pathway Intermediates | Total $^{14}C$ Metabolized |
|---|---|---|---|---|---|---|---|---|---|
| | Released $CO_2$ | Glycolate | Glyoxylate | Glycine | Serine | Glycerate | Neutral compounds | | |
| | *nmoles* | | | | | | | | |
| Glycolate-1-$^{14}C$ | 72 | | 7.6 | 35.0 | 55.0 | 8.5 | 191 | 106 | 1,330 |
| + α-HPMS | 30 | | 9.1 | 8.7 | 14.0 | 19.0 | 21 | 51 | 565 |
| + INH | 81 | | 14.0 | 42.0 | 67.0 | 18.0 | 170 | 141 | 1,270 |
| + glycine | 68 | | 9.1 | 60.0 | 62.0 | 25.0 | 187 | 156 | 1,180 |
| Glycine-1-$^{14}C$ | 67 | 1.8 | 1.4 | | 197.0 | 5.4 | 75 | 206 | 793 |
| + α-HPMS | 45 | 18.0 | 2.1 | | 269.0 | 3.5 | 36 | 293 | 716 |
| + INH | 60 | 2.5 | 1.7 | | 66.0 | 6.8 | 98 | 78 | 788 |
| + glycolate | 54 | 1.3 | 2.1 | | 110.0 | 8.5 | 110 | 122 | 807 |

cine-1-$^{14}C$ was added together with α-HPMS, 48% of the $^{14}C$ in the glycolate was in the carboxyl-carbon atom, suggesting the equally labeled carbons were produced by refixation of $^{14}CO_2$ (2).

## DISCUSSION

In these experiments metabolic pools were labeled by supplying $^{14}C$-substrates and the subsequent fate of the $^{14}C$ was followed. Such experiments are helpful in demonstrating the presence or absence of postulated pathways, although this technique often cannot be used to determine whether one pathway predominates over another under different conditions. From measurements of the total $^{14}CO_2$ released and the total $^{14}C$ metabolized, one can calculate that the added glycolate-1-$^{14}C$ was assimilated at a rate of about 12 μmoles/mg chlorophyll·hr. Thus no more than about 20% of the rate of the usual photorespiratory reactions was contributed by the trace quantities of labeled substrate added to the leaf tissue. The total $^{14}C$ metabolized was also not changed by any of the treatments except the addition of α-HPMS with glycolate-1-$^{14}C$ (Table II).

The site of the $^{14}CO_2$ evolution within the cell will help determine the proportion of the evolved $^{14}CO_2$ that is released compared to the amount refixed in the chloroplasts. If photorespiratory $^{14}CO_2$ produced from the decarboxylation of glycolate-1-$^{14}C$ arises in the chloroplasts (24), a large part of the evolved $^{14}CO_2$ would obviously be refixed in the light. However, if $^{14}CO_2$ derived from glycine-1-$^{14}C$ is produced in the mitochondria (12), there would be a greater diffusive resistance to refixation and a larger portion of the $^{14}CO_2$ evolved might be expected to leak out of the tissue and be measured. This may explain why more $^{14}C$ appeared to be metabolized into carbohydrate from glycolate than from glycine, relative to the $^{14}CO_2$ released. There may be greater refixation of $^{14}CO_2$ arising from glycolate-1-$^{14}C$ as discussed above.

Glycolate-1-$^{14}C$ can be decarboxylated without being converted into glycine (Fig. 1) because the addition of INH or nonradioactive glycine does not inhibit $^{14}CO_2$ release or the labeling of the glycolate pathway or carbohydrate itself. Added glycine-1-$^{14}C$ appears to have the potential for being decarboxylated as rapidly as added glycolate-1-$^{14}C$ by leaf tissue. However, glycine decarboxylation is not required for photorespiration because INH does not greatly change the $^{14}CO_2$ released or the radioactivity in carbohydrate from labeled glycine although it greatly inhibits the conversion of glycine to serine. These results show that the production of $CO_2$ is not essential in the glycolate pathway during the synthesis of serine from glycine as shown in Figure 1.

These data raise the question of the explanation for the observed inhibition of photorespiration caused by INH as shown in Table I and by Goldsworthy (3) in the absence of added substrates. In *Chlorella* this inhibitor also blocked the conversion of glycine to serine and caused glycine and glycolate to accumulate (10). INH does not inhibit glycolate oxidase activity; thus it probably blocks photorespiration in leaves by interfering with the synthesis of glycolate[2] or by reacting chemically with glyoxylate, thus slowing its decarboxylation. These alternatives have not yet been tested experimentally.

Several possibilities can be offered to explain the labeling of carbohydrate by the glycolate pathway (11, 15) without the necessity of a $CO_2$-producing reaction as illustrated in Figure 1. Some glycine may be decarboxylated directly without involving serine formation. Also glyoxylate may be transaminated with serine (thus bypassing glycine) to yield hydroxypyruvate in leaves (6). A transamination reaction between glycine and hydroxypyruvate to form serine is known to be catalyzed by leaf extracts (16), and this would result in a glycine to serine conversion in the absence of the serine hydroxymethyltransferase reaction. Even the latter reaction sequence may produce a C-1 fragment at the formaldehyde level and need not yield $CO_2$.

The results in Table II provide additional evidence beyond the points raised in the Introduction which suggest that the glycolate pathway at best may account for only a low level of $CO_2$ production during the formation of serine from glycine. This slow rate of $CO_2$ evolution may be fairly constant in all species in the light, even those such as maize that have low rates of photorespiration. In fact a more active glycolate pathway might diminish photorespiration by removing glyoxylate before it can be decarboxylated. Those species that have high rates of photorespiration therefore probably evolve

[2] After submitting this manuscript I have found that INH inhibits the synthesis of glycolate in tobacco leaves.

their vast quantitites of photorespiratory $CO_2$ by a more direct decarboxylation of glyoxylate as pictured in Figure 1.

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