

The Importance of Glyoxylate in Amino Acid Biosynthesis in Plants

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(Received 8 December 1964)

1. [$^{14}\text{C}_2$]Glyoxylate was rapidly metabolized by carrot storage tissues, pea leaves, pea cotyledons, sunflower cotyledons, corn coleoptiles, corn roots and pea roots. In many tissues over 70% of the supplied [$^{14}\text{C}_2$]glyoxylate was utilized during the 6 hr. experimental periods. 2. In all tissues, the chief products of [$^{14}\text{C}_2$]glyoxylate metabolism were carbon dioxide, glycine and serine. In several of the tissues, there was also a considerable incorporation of the label into the organic acids, particularly into glycollate. 3. Degradations of the labelled serine produced during [$^{14}\text{C}_2$]glyoxylate metabolism showed that glyoxylate carbon was incorporated into all three positions of the serine molecule. 4. The results are interpreted as indicating that glyoxylate is utilized by the tissues by pathways involving transamination, transmethylation, reduction and oxidative decarboxylation of the supplied glyoxylate.

Recent studies with micro-organisms and higher-plant tissues have indicated that glyoxylate plays an important role in intermediary metabolism. For example, the glyoxylate cycle is of widespread occurrence in bacteria and fungi grown on acetate media (Kornberg & Elsdén, 1961). Studies with germinating fatty seeds (Bradbeer & Stumpf, 1959; Carpenter & Beevers, 1959; Marcus & Velasco, 1960; Yamamoto & Beevers, 1960; Canvin & Beevers, 1961) have also provided considerable evidence for the operation of a glyoxylate cycle during the conversion of fat into carbohydrate. In these higher plants, glyoxylate is involved in organic acid biosynthesis, particularly in the synthesis of malate. Organic acids synthesized by this pathway are then utilized in sugar formation by a reversal of the glycolytic pathway (Beevers, 1961a).

Glyoxylate is also known to be produced in photosynthesizing tissues. Towers & Mortimer (1956) reported the formation of labelled glyoxylate during photosynthetic carbon dioxide fixation in a variety of plant tissues. Relatively high concentrations of this aldehyde acid have also been demonstrated to occur in wheat seedlings (Krupka & Towers, 1958a). These workers suggest glyoxylate might arise from the breakdown of allantoin in these tissues (Krupka & Towers, 1958b). Further, studies with [$^{14}\text{C}_2$]glyoxylate showed that this compound was readily converted into glycine by various parts of wheat seedlings (Krupka & Towers, 1959).

Studies of glycollate metabolism in a variety of leaf tissues by Rabson, Tolbert & Kearney (1962) have suggested that oxidation to glyoxylate is an

important reaction in the conversion of glycollate into hexoses. These workers have presented considerable evidence from short-term experiments that glycollate, arising from photosynthetic carbon dioxide fixation, is converted into glycerate and hexoses, by a pathway involving the intermediary formation of glyoxylate, glycine and serine.

Detailed studies of glyoxylate metabolism in illuminated wheat leaves by Wang & Waygood (1962) have also emphasized the importance of glyoxylate in glycine biosynthesis. These workers implicate glyoxylate in amino acid and carbohydrate biosynthesis by operation of a glyoxylate \rightarrow glycine \rightarrow serine \rightarrow carbohydrate pathway. Further support for the operation of this pathway has been derived from experiments where intermediates in the reaction sequence have been fed to the tissues (Wang & Burris, 1963).

Investigations of glycine metabolism in non-green plant tissues by Sinha & Cossins (1964a) showed that glyoxylate was readily produced during metabolism of this amino acid. Further, it was suggested that glyoxylate might play an important role in serine biosynthesis. To investigate these possibilities, experiments have been performed with [$^{14}\text{C}_2$]glyoxylate in an attempt to assess the importance of this compound in amino acid biosynthesis.

In the present investigation, a variety of plant tissues, including germinating cotyledons, roots, coleoptiles, leaves and storage tissues, have been examined for ability to metabolize [$^{14}\text{C}_2$]glyoxylate. In all experiments, carbon dioxide, glycine and serine were the chief products of glyoxylate metabolism. Degradations of the serine produced

from glyoxylate have indicated that all three carbon atoms of this amino acid can be derived from glyoxylate.

MATERIALS AND METHODS

Plant materials. Seeds of pea (*Pisum sativum* L. var. Homesteader), corn (*Zea mays* L. var. Falconer) and sunflower (*Helianthus annuus* L. var. Mammoth Russian) were soaked overnight in tap water at 25° and then germinated at 25° in dishes containing moist vermiculite. After germination for 3 days, root tips (5 mm. in length) were excised from the corn and pea seedlings. Similarly, 5 mm. sections of coleoptiles were excised from samples of 4-day-old corn seedlings. Cotyledons of sunflower seeds were used in the feeding experiments after soaking samples of the seeds overnight in tap water. Cotyledons of pea seeds were excised from 3-day-old seedlings. In experiments with mature pea leaves, these tissues were removed from 15-day-old seedlings grown at 25° in normal daylight. The storage tissues of carrot (*Daucus carota* L.) were purchased locally and stored at 5° before use in the feeding experiments.

Feeding experiments. To facilitate the penetration of the small amounts of glyoxylate supplied in the feeding experiments, the tissues were sliced as previously described (Cossins & Beever, 1963; Sinha & Cossins, 1964a).

[¹⁴C₂]Glyoxylate was purchased from California Corp. for Biochemical Research (Los Angeles, Calif., U.S.A.) and was diluted with carrier glyoxylate to give solutions with final specific activities of 2 μC/μmole and 1 μC/μmole respectively/0.1 ml. of solution.

Samples of the tissue (0.5 g. fresh wt.) were incubated in Warburg vessels (20 ml. capacity) at 30° in the dark with labelled glyoxylate and sodium phosphate buffer as shown in the Tables. In all experiments, the centre wells contained 0.2 ml. of carbonate-free 20% (w/v) NaOH solution to absorb carbon dioxide evolved during the experimental periods. The absorbed carbon dioxide was converted into BaCO₃ at the end of the experimental periods and plated on to glass-fibre disks before being assayed for ¹⁴C content in a gas-flow counter with 10% efficiency (model C110B; Nuclear-Chicago Corp., Chicago, U.S.A.) as described by Sinha & Cossins (1964a). The counts were corrected for self-absorption and background.

Analytical methods. At the end of the experimental periods, the tissues were killed by the addition of 15 ml. of boiling 80% (v/v) ethanol and ground finely in a hand-operated blender. The procedure of extraction and fractionation of the extracts was as described by Cossins & Beever (1963). This involved separation of the water-soluble materials into four fractions, namely acidic amino acids, neutral and basic amino acids, organic acids and sugars, by the use of ion-exchange resins (Canvin & Beever, 1961).

Further fractionation of the organic acids was achieved by gradient elution of the acids from Dowex 1 (X10; formate form) by the method of Palmer (1955). The individual organic acids separated by this procedure were identified by their position of elution from the formate resin and by co-chromatography with authentic organic acids (Sinha & Cossins, 1964a). Labelled oxalic acid was further identified by preparation of the calcium salt as described by Miller, Morton & Wells (1963).

Radioactive components of the neutral and basic amino

acid extracts were separated by two-dimensional descending paper chromatography with phenol-water (8:3, v/v) followed by butan-1-ol-acetic acid-water (4:1:5, by vol.) as solvent systems. Separation of glycine and serine was achieved by rechromatography of the glycine-serine area, eluted from the paper chromatograms, in butan-1-ol-acetone-water-diethylamine (20:20:10:3, by vol.).

Radioactive areas on the paper chromatograms were detected by radioautography with Kodak No-Screen X-ray film and by scanning with a Nuclear-Chicago 4Pi Actigraph (model no. 4502), and assayed in a gas-flow counter after elution and drying on nickel-plated planchets as described by Sinha & Cossins (1964a).

Determination of ¹⁴C in the insoluble residue. After the extraction of water-soluble materials, the insoluble residues were dried thoroughly at 100°. Samples of this material were then subjected to acid hydrolysis according to the method described by Ranjan & Laloraya (1960). The hydrolysate was then filtered and dried *in vacuo* at 40°. Products of hydrolysis were dissolved in 10 ml. of water, and 0.1 ml. samples were dried on nickel-plated planchets and assayed for ¹⁴C in a gas-flow counter as described by Sinha & Cossins (1964a).

Degradation of labelled serine samples. Samples of [¹⁴C]serine recovered from the tissues after feeding with [¹⁴C₂]glyoxylate were degraded by using periodate (Sakami, 1950). The products of periodate oxidation were recovered as [¹⁴C]formaldehyde dinitrophenylhydrazone and sodium [¹⁴C]formate, which were plated on to nickel-plated planchets and assayed in a gas-flow counter as described by Sinha & Cossins (1964a). The counts were corrected for self-absorption and background.

RESULTS

Metabolism of glyoxylate by excised root tips. In the experiment summarized in Table 1, glyoxylate metabolism was investigated in root tips excised from pea and corn seedlings. In both cases, glyoxylate carbon was incorporated into all the fractions isolated. Considerable amounts of radioactivity were incorporated into the carbon dioxide released during the experimental period. Further, the organic acid and amino acid fractions were important products of glyoxylate metabolism. In both the tissues examined, glycine and serine together contained the bulk of the ¹⁴C incorporated into the amino acid fraction.

Metabolism of glyoxylate by corn coleoptiles and pea leaves. Glyoxylate was readily metabolized by corn coleoptiles and pea leaves (Table 2). In pea leaves, there was considerable oxidation of glyoxylate to carbon dioxide in addition to incorporation of glyoxylate carbon into glycine and serine. The feeding of glyoxylate to corn coleoptiles resulted in a major incorporation of glyoxylate carbon into the organic acids. In addition to this, glyoxylate was utilized for the synthesis of glycine and serine and was oxidized to carbon dioxide.

Metabolism of glyoxylate by germinating seeds. The results of glyoxylate-feeding experiments with

Table 1. *Metabolism of [¹⁴C₂]glyoxylate by excised root tips*

The root tips (5 mm.) were excised from 3-day-old seedlings, and 0.5 g. samples incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer, pH 5.6, and 1 μmole of glyoxylate (containing 1 μC of ¹⁴C) in a total volume of 0.6 ml.

Fraction	Pea roots		Corn roots	
	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)
Carbon dioxide	55600	24.2	60000	30.4
Lipids	3200	1.4	8800	4.5
Sugars	3400	1.4	10400	5.2
Organic acids	87200	38.0	16700	8.4
Amino acids				
Glycine	14500	6.3	29000	14.7
Serine	12400	5.4	19000	9.9
Glutamic acid + aspartic acid	20200	8.8	13400	7.0
Others	10200	4.4	—	—
Residue	22600	9.8	39500	20.5
Total ¹⁴ C incorporated	229000		197000	

Table 2. *Metabolism of [¹⁴C₂]glyoxylate by corn coleoptiles and pea leaves*

Corn coleoptiles were excised from 4-day-old seedlings and pea leaves from 15-day-old seedlings, and 0.5 g. samples of tissue slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer, pH 5.6, and 1 μmole of glyoxylate (containing 1 μC of ¹⁴C) in a total volume of 0.6 ml.

Fraction	Corn coleoptiles		Pea leaves	
	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)
Carbon dioxide	29700	9.1	103200	23.1
Lipids	1000	0.3	1400	0.3
Sugars	5500	1.7	16000	3.7
Organic acids	192000	58.8	20700	4.6
Amino acids				
Glycine	37700	11.6	170000	38.1
Serine	21500	6.6	63200	14.2
Glutamic acid + aspartic acid	6000	1.8	1800	0.4
Others	10500	3.2	44000	9.9
Residue	22600	6.9	25700	5.7
Total ¹⁴ C incorporated	326500		446000	

pea and sunflower cotyledons (Table 3) give results very similar to those shown in Tables 1 and 2. For example, in these germinating seeds, there was considerable metabolism of the supplied glyoxylate, and after a 6 hr. experimental period carbon dioxide, the organic acids and the amino acids all contained considerable amounts of ¹⁴C. As in the preceding experiments, large amounts of radioactivity were present in glycine and serine as a result of [¹⁴C₂]-glyoxylate metabolism.

Metabolism of glyoxylate by carrot tissue slices. In studies of glycine metabolism in carrot storage tissues, Sinha & Cossins (1964a) reported a major conversion of glycine into carbon dioxide, serine, glyoxylate and glycollate. It was suggested that transamination to glyoxylate and oxidation to carbon dioxide were major reactions during the

metabolism of glycine by these tissues. In the present studies, [¹⁴C₂]glyoxylate was supplied to carrot tissues (Table 4) and the distribution of ¹⁴C in the products of glyoxylate metabolism was examined after a 6 hr. experimental period. The results are similar to those reported previously for feeding with [¹⁴C₂]glycine, namely that carbon dioxide, the organic acids, serine, glycine and the insoluble residue are all important products of glyoxylate metabolism.

In the presence of 10 μmoles of formate, this distribution of ¹⁴C from [¹⁴C₂]glyoxylate was slightly altered. This treatment resulted in decreases in the amounts of ¹⁴C being incorporated into carbon dioxide, glycine and the insoluble residue, but led to increases in the radioactivities of serine and the organic acid fraction.

Table 3. *Metabolism of [¹⁴C₂]glyoxylate by germinating seeds*

Sunflower cotyledons were excised from 1-day-old seedlings and pea cotyledons from 3-day-old seedlings, and 0.5g. samples of cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer, pH 5.6, and 1 μmole of glyoxylate (containing 1 μC of ¹⁴C) in a total volume of 0.6 ml.

Fraction	Pea cotyledons		Sunflower cotyledons	
	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)
Carbon dioxide	78 200	26.4	55 000	14.7
Lipids	3 300	1.1	2 500	0.67
Sugars	3 000	1.0	9 000	2.4
Organic acids	118 700	40.0	67 500	18.1
Amino acids				
Glycine	22 600	7.6	39 600	10.6
Serine	8 300	2.8	39 800	10.7
Glutamic acid + aspartic acid	25 400	8.5	47 000	12.7
Others	14 000	4.7	17 000	4.5
Residue	22 600	7.6	55 000	14.7
Total ¹⁴ C incorporated	296 100		371 600	

Table 4. *Effect of formate on the metabolism of [¹⁴C₂]glyoxylate by carrot tissue*

Slices of storage tissue (0.5g.) were incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer, pH 5.6, and 1 μmole of glyoxylate (containing 1 μC of ¹⁴C) in a total volume of 0.6 ml., and the effects of 10 μmoles of added formate determined.

Fraction	[¹⁴ C ₂]Glyoxylate added		[¹⁴ C ₂]Glyoxylate + 10 μmoles of formate added	
	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)	(¹⁴ C counts/min.)	(% of incorporated ¹⁴ C)
Carbon dioxide	104 300	31.2	95 000	29.6
Lipids	4 500	1.3	2 600	0.8
Sugars	3 200	1.0	2 000	0.6
Organic acids	84 500	25.3	90 000	28.0
Amino acids				
Glycine	26 700	8.0	24 000	7.4
Serine	20 600	6.2	22 000	7.0
Glutamic acid + aspartic acid	8 000	2.4	9 400	3.0
Residue	82 700	24.7	76 000	23.7
Total ¹⁴ C incorporated	334 000		321 000	

In other experiments with carrot tissue slices, the time of incubation of the tissues with the labelled glyoxylate solutions was decreased. After short periods of [¹⁴C₂]glyoxylate metabolism, the tissues were killed and extracted as described in the Materials and Methods section. The results of these experiments are summarized in Table 5; they show that, as the time of incubation of the tissues with [¹⁴C₂]glyoxylate decreased, the number of compounds containing ¹⁴C was also decreased. After only 2 min. of glyoxylate metabolism, glycollate and glycine were the chief products. With increases in the experimental period, radioactivity was detected in serine, and after 30 min. radioactivity was detected in all the fractions isolated.

Incorporation of [¹⁴C₂]glyoxylate into the organic acids of plant tissues. The results presented in

Tables 1-5 show that the organic acid fraction generally contains large amounts of radioactivity as a result of feeding with [¹⁴C₂]glyoxylate. The radioactive components of this fraction were further separated by elution from Dowex columns as described in the Materials and Methods section. The distribution of ¹⁴C in the organic acids separated is shown in Table 6. The amounts of ¹⁴C present in the individual organic acids varied considerably in the different tissues examined. However, in most tissues, glycollate and oxalate contained large percentages of the ¹⁴C incorporated into the organic acid fraction. In carrot tissues and pea cotyledons, considerable amounts of glyoxylate carbon were also incorporated into malate, citrate and succinate. Additions of formate to carrot tissues metabolizing [¹⁴C₂]glyoxylate increased the incorporation of the

Table 5. *Sequence of incorporation of ^{14}C into products of [$^{14}\text{C}_2$]glyoxylate metabolism in carrot tissue slices*

Slices (0.5 g.) of carrot storage tissue were incubated at 30° in darkness with 100 μmoles of phosphate buffer, pH 5.6, and 1 μmole of glyoxylate (containing 2 μC of ^{14}C) in a total volume of 0.6 ml.

Fraction	Time of incubation.....2 min.		5 min.		15 min.		30 min.	
	(^{14}C counts/min.)	(% of incor- porated ^{14}C)	(^{14}C counts/min.)	(% of incor- porated ^{14}C)	(^{14}C counts/min.)	(% of incor- porated ^{14}C)	(^{14}C counts/min.)	(% of incor- porated ^{14}C)
Carbon dioxide	900	0.8	900	0.7	900	0.6	1900	1.0
Lipids	3100	2.7	2300	1.7	2800	1.9	5400	3.0
Sugars	Not active	—	1000	0.7	1000	0.7	1000	0.7
Organic acids								
Glycollate	76000	67.9	95000	69.8	95000	63.1	104500	58.4
Succinate	Not active	—	Not active	—	Not active	—	3500	1.9
Malate	Not active	—	Not active	—	Not active	—	2500	1.4
Others	Not active	—	5000	3.7	5000	3.4	3500	1.9
Amino acids								
Glycine	22400	20.0	21700	16.0	29000	19.5	40000	22.3
Serine	Not active	—	Not active	—	2000	1.4	4000	2.2
Glutamic acid + aspartic acid	2600	2.3	2600	1.9	5500	3.7	3300	1.8
Residue	7000	6.2	7500	5.5	8300	5.6	10000	5.6

Table 6. *Metabolism of [$^{14}\text{C}_2$]glyoxylate by plant tissues: organic acid fraction*

The experimental procedure was as described in Tables 1-4. The results are expressed as percentages of ^{14}C incorporated into the organic acid fractions.

Organic acids	Carrot storage tissues						[$^{14}\text{C}_2$]Glyoxylate added	[$^{14}\text{C}_2$]Glyoxylate + 10 μmoles of formate added
	Pea leaves	Sunflower cotyledons	Corn coleoptiles	Pea cotyledons	Corn roots			
Glycollate	18.1	25.1	80.2	55.2	62.3	8.0	18.1	
Oxalate	13.0	55.5	8.1	7.6	16.1	17.0	8.9	
Malate	6.3	4.5	3.8	24.9	8.4	23.9	29.7	
Succinate	5.6	—	—	9.9	13.2	23.9	18.9	
Citrate	21.2	14.9	—	2.3	—	6.7	6.4	
Glucose phosphate	—	—	—	—	—	13.6	12.5	
Pyruvate	—	—	—	—	—	6.7	5.5	
Others	35.7	—	7.8	—	—	—	—	

Table 7. *Intramolecular distribution of ^{14}C in serine after feeding with [$^{14}\text{C}_2$]glyoxylate*

Samples of [$^{14}\text{C}_3$]serine recovered from tissues after 6 hr. of [$^{14}\text{C}_2$]glyoxylate metabolism were degraded by using periodate as described in the Materials and Methods section. The results are expressed as percentages of the ^{14}C recovered from products of the degradation procedures. The sunflower cotyledons were excised from 1-day-old seedlings and the pea leaves from 15-day-old seedlings.

Carbon no.	Carrot storage tissue		Sunflower cotyledons [$^{14}\text{C}_2$]Glyoxylate added	Pea leaves [$^{14}\text{C}_2$]Glyoxylate added	
	[$^{14}\text{C}_2$]Glyoxylate added	[$^{14}\text{C}_2$]Glyoxylate + 10 μmoles of formate added			
CO ₂ H	1	52.8	54.5	33.3	60.8
 CH·NH ₂	2	42.4	43.3	38.8	19.3
 CH ₂ ·OH	3	4.7	2.1	27.7	19.8

label into glycollate without appreciably affecting the amounts of ^{14}C detected in malate, citrate and succinate.

Intramolecular distribution of ^{14}C in serine. Table 7 shows the percentage distribution of ^{14}C in serine samples extracted from the plant tissues after periods of $[^{14}\text{C}_2]$ glyoxylate metabolism. It is clear from the results obtained that ^{14}C from labelled glyoxylate was incorporated into all three positions of the serine molecule. In the pea leaves and sunflower cotyledons, there was a considerable incorporation of ^{14}C into C-3 of serine. In contrast, serine extracted from carrot tissues after $[^{14}\text{C}_2]$ glyoxylate metabolism contained only small amounts of radioactivity in C-3. This labelling of C-3 was decreased in the presence of $10\ \mu\text{moles}$ of formate.

DISCUSSION

The results of the present experiments clearly show that all the plant tissues examined possessed the ability to metabolize the micromolar amounts of $[^{14}\text{C}_2]$ glyoxylate supplied. In some tissues, nearly all the $[^{14}\text{C}_2]$ glyoxylate supplied was utilized during the experimental periods (Table 8).

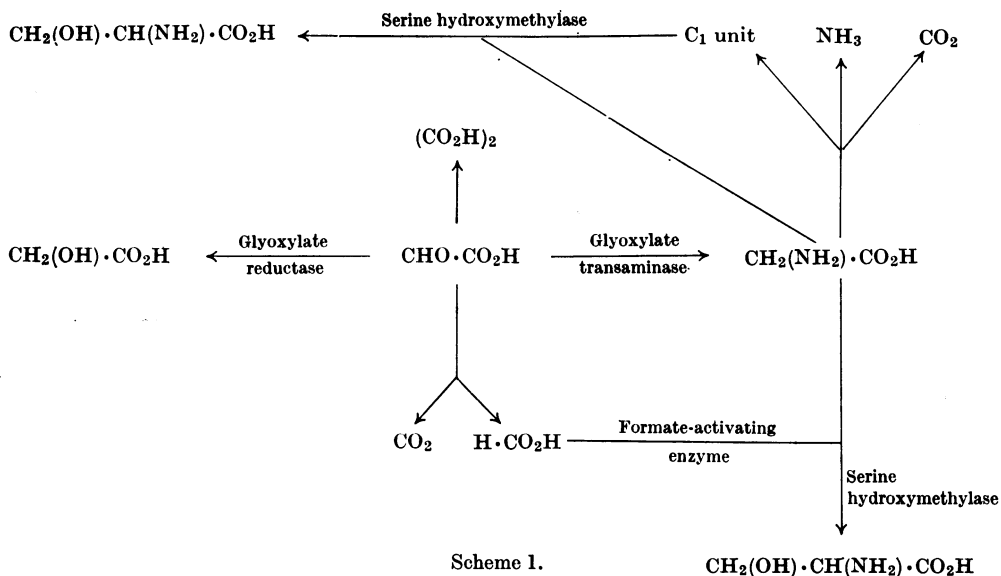
This metabolism of glyoxylate was in all cases associated with labelling of glycine and serine in addition to the organic acids. These compounds are also important products of $[^{14}\text{C}_2]$ glyoxylate metabolism in germinating fatty seeds (Cossins & Sinha, 1964a; Sinha & Cossins, 1964b). The production of glycine from glyoxylate probably involves a transamination reaction. There is considerable evidence for the widespread occurrence of glyoxylate transaminase in animals, micro-organisms and higher plants (Nakada, 1964; McCurdy & Cantino, 1960; Campbell, 1956; Sastry & Ramakrishnan, 1961; Cossins & Sinha, 1964b). Studies of this enzyme system in extracts of the tissues used in the present investigation have indicated that the enzyme-catalysed reaction is reversible but favours the formation of glycine *in vitro* (Cossins & Sinha, 1964b).

The production of serine from glyoxylate probably involves a series of enzyme-catalysed reactions: first, the formation of glycine from glyoxylate by a transamination reaction; secondly, the glyoxylate molecule is split to yield an activated C_1 unit that condenses with glycine to yield serine labelled in all three positions of the molecule. The production of formate from glycollate has been demonstrated in extracts prepared from higher-plant tissues (Tolbert, Clagett & Burris, 1949). The reaction involves the intermediary formation of glyoxylate and then formate is produced from the aldehyde group of this acid. The production of formate from glyoxylate has also been demonstrated in animal tissues (Nakada, Friedmann & Weinhause, 1955; Nakada & Sund, 1958). Experiments with $[^{14}\text{C}]$ formate (Tolbert, 1955; E. A. Cossins & S. K. Sinha, unpublished work) have clearly shown that plant tissues can incorporate this compound into C-3 of serine. This reaction probably involves a formate-activating enzyme and serine hydroxymethylase, which are involved in similar reactions in animal tissues (Friedkin, 1963).

Some support for these suggestions about the formation of serine from glyoxylate comes from the effects of formate on glyoxylate metabolism. In carrot tissues, additions of formate led to slight increases in the amounts of radioactivity present in serine (Table 4) but also decreased the amounts of ^{14}C entering C-3 of serine (Table 7). However, the C_1 unit required to provide C-3 of serine might be derived more readily from glycollate (Tolbert & Cohan, 1953) or from C-2 of glycine (Richert, Amberg & Wilson, 1962; Pitts & Crosbie, 1962). As glycollate and glycine were both heavily labelled after $[^{14}\text{C}_2]$ glyoxylate metabolism, they might also yield labelled C_1 units to be used in the synthesis of serine. Experiments with plant tissue extracts (S. K. Sinha & E. A. Cossins, unpublished work) have shown that C_1 units for synthesis of serine can arise from C-2 of glycine. Free glyoxylate does not appear to be an intermediate in this reaction, which is dependent on tetrahydrofolate. Clearly,

Table 8. Percentage of $[^{14}\text{C}_2]$ glyoxylate utilized by higher-plant tissues

Tissue	$10^{-5} \times$ Radioactivity in $[^{14}\text{C}_2]$ glyoxylate supplied (counts/min.)	$10^{-5} \times$ Radioactivity recovered in products after 6 hr. (counts/min.)	Percentage of $[^{14}\text{C}_2]$ glyoxylate utilized
Carrot storage tissue	4.5	3.34	74
Pea leaves	4.5	4.46	99
Pea cotyledons	3.5	2.96	84
Sunflower cotyledons	4.5	3.71	82
Corn coleoptiles	4.5	3.26	72
Corn roots	3.5	1.97	56
Pea roots	3.0	2.29	76



if these reactions occur in the tissues examined for [$^{14}\text{C}_2$]glyoxylate metabolism, ^{14}C will be distributed throughout the serine molecule (Table 7).

Besides extensive labelling of glycine and serine, all of the tissues produced some labelled glycollate after feeding with [$^{14}\text{C}_2$]glyoxylate (Table 6). In some tissues, e.g. in corn coleoptiles, corn roots and pea cotyledons, this acid contained the bulk of the ^{14}C incorporated into the organic acid fraction. Glycollate can be readily produced from glyoxylate by the action of glyoxylate reductase, which is of widespread occurrence in plants (Stafford, Magaldi & Vennesland, 1954; Zelitch & Ochoa, 1953; Zelitch, 1955).

In addition to the labelling of glycollate, some of the tissues contained appreciable amounts of radioactivity in oxalate. Although very little is known of the pathway for oxalate formation in plants, studies with *Oxalis pes-caprae* by Millerd *et al.* (1963) have indicated that glycollate and glyoxylate are the immediate precursors of this acid. It is clear therefore that the labelling of oxalate that occurs in the present experiments might be explained if glycollate and glyoxylate are precursors of oxalate in these tissues also. The amounts of ^{14}C incorporated into oxalic acid during the experimental periods might reflect the relative activities of the enzymes catalysing its formation from glyoxylate and the relative pool sizes of oxalate in the tissues examined.

Labelling of malate, citrate and succinate occurred in many of the tissues as a result of feeding with [$^{14}\text{C}_2$]glyoxylate (Table 6). Labelling of malate might occur as a result of malate-

synthetase activity, but there is considerable evidence (Beever, 1961*a,b*) that this enzyme is mainly restricted to plant tissues that are metabolizing fat. It is probable therefore that the labelling of malate observed in the present work was not due to malate-synthetase activity. Labelling of citrate, malate and succinate could conceivably occur if the labelled serine produced from [$^{14}\text{C}_2$]glyoxylate was metabolized via the tricarboxylic acid cycle. Alternatively, labelling of these organic acids would occur if $^{14}\text{CO}_2$ produced from glyoxylate were assimilated during the experimental periods by a carboxylation reaction (Walker, 1962).

From the present experiments, it appears that glyoxylate metabolism in the plant tissues examined involves several distinct metabolic reactions, as summarized in Scheme 1. Metabolism of [$^{14}\text{C}_2$]glyoxylate by these pathways would lead to a distribution of ^{14}C among the major products isolated during the present work.

This work was supported by a grant-in-aid of research from the National Research Council of Canada.

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