The Metabolism of [^{14}C]Glycine by Plant Tissues

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Apart from playing an important role as a common constituent in plant proteins, glycine is known to be involved in several important reactions of intermediary metabolism. Glycine can enter into organic acid metabolism by transamination reactions which are of widespread occurrence in microorganisms and plant tissues. For example, Campbell (1956) has reported the presence of glyoxylate–glycine transaminase systems from *Pseudomonas* and *Neurospora*. Similar findings were reported by McCurdy & Cantino (1960) for extracts prepared from the fungus *Blastocladiella*. In higher-plant tissues, the conversion of glycine into glyoxylic acid is generally associated with transaminase activity (Leonard & Burris, 1947; Wilson, King & Burris, 1954). In *Diplococcus glycinophilus*, the glycine molecule is directly cleaved into carbon dioxide and a unit of one carbon atom without the intermediary formation of an acid with two carbon atoms (Sagers & Gunsalus, 1961).

Although considerable information has been obtained about the role of glycine in the biosynthesis of serine in animal tissues (reviewed by Sakami, 1955), glycine–serine interconversions have not been extensively studied in plant tissues. In wheat plants, McConnell & Bilinski (1959) have demonstrated the synthesis of [^{14}C]serine from [1-^{14}C]-glycine and [^{14}C]formate. Wang & Burris (1963) have clearly demonstrated that serine is among the first compounds labelled in experiments where [2-^{14}C]glycine was fed to wheat leaves in the light. E. A. Cossins & S. K. Sinha (unpublished work) have observed a greater incorporation of [^{14}C]-formate into serine by radish-leaf slices when micromolar amounts of glycine are added during the feeding experiments.

Wang & Waygood (1962) have examined the pathway by which glycine is involved in carbohydrate biosynthesis in illuminated wheat leaves. From evidence derived from isotopic competition studies and from the position of ^{14}C in glucose, derived from [2-^{14}C]glycine, they implicate serine and hydroxypyruvate as intermediates. Glucose was apparently formed by a condensation of two C_3 units by a reversal of glycolysis.

Investigations on glycine metabolism in higher plant materials have therefore been largely conducted with illuminated chlorophyll-containing tissues. As several investigations on the path of carbon in photosynthesis (Buchanan *et al.* 1952; Towers & Mortimer, 1956) have demonstrated that glycine, glycollate and serine are among the early products of carbon dioxide assimilation in the light, this might have important effects on the pathway for glycine metabolism in these tissues.

The present paper describes experiments on the metabolism of [$^{14}C_2$]glycine in non-green plant tissues, namely slices of castor-bean endosperm and slices of carrot tissues. Castor-bean endosperm slices were selected because considerable information is available concerning the formation and metabolism of glyoxylate in these tissues (Carpenter & Beevers, 1959; Yamamoto & Beevers, 1960). Carrot tissues form large amounts of [^{14}C]serine when supplied with [^{14}C]methanol (Cossins & Beevers, 1962). These tissues were therefore examined in the present investigation to ascertain whether [^{14}C]glycine was involved in serine biosynthesis.

MATERIALS AND METHODS

Plant materials. Seeds of the castor bean (*Ricinus communis* L. var. *zanzibarensis*) were soaked overnight in distilled water at 25°. After this soaking period the seeds were transferred to moist vermiculite and allowed to germinate for 5 days at 30°. The storage tissues of carrot (*Daucus carota* L.) were purchased locally and stored at 5° before use in the feeding experiments.

Feeding experiments. In all experiments the plant materials were sliced to facilitate the penetration of the small amounts of glycine supplied in the feeding experiments. The preparation of the tissue was as described by Cossins & Beevers (1963). In the present experiments, 1 mm.-thick slices of 5-day-old castor-bean endosperm, and slices, 6 mm. diam. and 1 mm. thick, of the cortical tissue of carrot roots were used.

[^{14}C]Glycine was purchased from Atomic Energy of Canada Ltd., Ottawa, and diluted with water to give final specific activity 2 μc/μmole/0·1 ml. of solution. Degradation studies (Table 6) indicated that 34 % and 66 % of the ^{14}C was present in the C-1 and C-2 positions respectively.

Slices (1 g. fresh wt.) of castor-bean endosperm and carrot tissues were incubated with 1 μmole of [$^{14}C_2$]glycine (radioactivity approx. 4×10^5 counts/min.). Incubations were carried out in Warburg vessels (20 ml. capacity) at 30° in the dark. In addition to the radioactive glycine solutions, the flasks contained 200–300 μmoles of tris–HCl buffer,

pH 7·2. In experiments where the effects of inhibitors were investigated, the slices were incubated in the buffer and inhibitor for a 30 min. period at 30° before addition of the radioactive glycine solutions.

Carbon dioxide evolved during the feeding experiments was absorbed in carbonate-free 20% (w/v) NaOH soln. added to the centre wells of the Warburg flasks. The absorbed carbonate was converted into $BaCO_3$ at the end of the experimental periods and filtered on to glass-fibre filter-paper disks. The disks were dried thoroughly at 100° mounted on nickel-plated planchets and assayed for radioactivity in a gas-flow counter with 10% efficiency (model C 110 B; Nuclear–Chicago Corp., Chicago, U.S.A.). The counts were corrected for self-absorption and background.

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

Further fractionation of the organic acids was achieved by gradient elution of the acids from Dowex 1 (X 10; formate form) by the method of Palmer (1955). Identity of the organic acids was established by their position of elution from the Dowex (formate form) resins and by co-chromatography with authentic organic acids in propan-1-ol–aq. NH_3 soln. (sp.gr. 0·88) (3:2, v/v), phenol–water (8:3, v/v) and butan-1-ol–acetic acid–water (4:1:5, by vol.). Glyoxylic acid was further identified by preparation of the 2,4-dinitrophenylhydrazones by the method of Turner & Quartley (1956).

The components of the neutral and basic amino acid extract were separated by two-dimensional descending paper chromatography in 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 two-dimensional paper chromatograms in the solvent system used by Hardy, Holland & Nayler (1955).

Radioactive areas on the paper chromatograms were located by radioautography with Kodak No-Screen X-ray film and by scanning with a Nuclear–Chicago 4 Pi Actigraph (model no. 4502). Radioactivity of the soluble fractions was assayed after plating portions (0·2 ml.) of the extracts on to nickel-plated steel planchets and drying under an infrared lamp. In all cases the radioactivities were corrected for background.

After thorough drying at 100°, samples of the insoluble residue were subjected to acid hydrolysis by the method described by Ranjan & Laloraya (1960) or were subjected to combustion to CO_2 (Van Slyke & Folch, 1940) in a Stutz & Burris (1951) apparatus.

Degradation of [^{14}C]*serine.* Samples of [^{14}C]serine isolated by the paper-chromatographic techniques described above were degraded by periodate oxidation, which yielded CO_2 from the carboxyl group and formate and formaldehyde from the C-2 and C-3 positions respectively (Sakami, 1950). For recovery of the products of the periodate oxidation, the method described by Sakami (1950) was modified as follows: after removal of the carboxyl group as CO_2, the reaction flask was cooled rapidly to 2° and the pH raised to 8 by addition of N-NaOH soln. The formaldehyde derived from the C-3 position of serine was then distilled off and collected in 0·5% 2,4-dinitrophenylhydrazine in 6 N-HCl. The 2,4-dinitrophenylhydrazones of formaldehyde were extracted into 10% ethanol in chloroform (Turner & Quartley, 1956) and radioactivity was determined by plating portions (0·2 ml.) of the ethanol–chloroform extract on metal planchets.

After removal of the formaldehyde by distillation the flask was cooled in ice and the contents were adjusted to pH 2 by additions of 20% phosphoric acid. The acidified solution was distilled once again and formic acid derived from the C-2 position of serine was collected in 2 N-NaOH soln. Samples (0·2 ml.) of the formate solution were plated on metal planchets and then counted for radioactivity.

When samples of [^{14}C]serine were degraded by this procedure, recoveries of radioactivity from the respective carbon atoms were within the range 90–95%.

Degradation of [^{14}C]*glycine.* Samples of [$^{14}C_2$]glycine used in the feeding experiments and samples separated from the tissues at the end of the experimental periods were degraded by using ninhydrin in citrate buffer (Van Slyke, Dillon, MacFadyen & Hamilton, 1941). This yielded CO_2 from the carboxyl group, which was trapped in 20% (w/v) KOH, converted into $BaCO_3$ and assayed for radioactivity as described above. In all cases, radioactivity of the C-2 position of the glycine molecule was calculated by difference between the total radioactivity of the sample and radioactivity recovered as CO_2 when the sample was treated with ninhydrin.

RESULTS

Metabolism of glycine by slices of castor-bean endosperm. During the 6 hr. experimental period, the slices of castor-bean endosperm metabolized approx. 46% of the [^{14}C]glycine supplied (Table 1).

Table 1. *Utilization of* [$^{14}C_2$]*glycine by castor-bean endosperm*

Endosperm slices (1 g.) of 5-day-old seedlings were incubated for 6 hr. at 30° in Warburg flasks containing: 300 μmoles of tris–HCl buffer, pH 7·2; 0·1 ml. of [$^{14}C_2$]glycine (2 μc/μmole), 326 000 counts/min. of ^{14}C. Total volume 1·6 ml. Tissues were killed and extracted as described in the text.

Fraction	Counts/min.	% of ^{14}C incorporated
Lipid	2 400	1
Amino acids		
Neutral and basic amino acids, serine	49 700	33
Acidic amino acids	23 300	15
Sugars	3 900	3
Organic acids		
Glyoxylate	6 760	4
Glycollate	2 050	1
Succinate	1 150	0·7
Malate	1 070	0·7
Citrate	460	0·3
Others	810	0·5
Carbon dioxide	47 600	31
Residue	12 500	8
Total ^{14}C incorporated	150 700	—
% of [$^{14}C_2$]glycine utilized	46	—

This utilization resulted in considerable amounts of radioactivity being incorporated into all the fractions isolated. The major products of glycine metabolism were serine and carbon dioxide, which accounted for 33 and 31 % respectively of the ^{14}C incorporated during the 6 hr. experimental period. In addition, radioactivity was present in the acidic amino acid and the organic acid fractions. Although the percentage of ^{14}C incorporated into the organic acids of castor-bean endosperm was less than that incorporated into the acidic amino acids, the label was mainly present in glycollate and glyoxylate. Smaller amounts of ^{14}C were obvious in malate, in succinate, in lipid material and in the sugars.

Metabolism of glycine by carrot tissues. After 6 hr. incubation with [$^{14}C_2$]glycine, the carrot tissues metabolized 99 % of the supplied ^{14}C (Table 2). The largest percentage of ^{14}C incorporated was found in the insoluble material. As in the castor-bean slices, considerable amounts of radioactivity were present in the carbon dioxide evolved and serine was the major radioactive component of the soluble fractions isolated.

Radioactivity of the organic acid extract (Table 3) was due mainly to large amounts of labelling in glyoxylate as occurred in the castor-bean endosperm. In addition, malate, succinate, citrate and the pyruvate fraction contained ^{14}C derived from glycine.

In experiments where the time of incubation with the [$^{14}C_2$]glycine solutions was reduced (Table 4) the distribution of activity in the various fractions was considerably altered. After 5 min. of glycine metabolism the organic acids were the main products. In contrast, only small amounts of glycine carbon were converted into carbon dioxide. As the time of incubation with the [$^{14}C_2$]glycine

Table 2. *Utilization of* [$^{14}C_2$]*glycine by carrot tissues*

Cortical tissues (1 g.) of carrot roots were incubated for 6 hr. at 30° in Warburg flasks containing: 200 μmoles of tris buffer, pH 7·2; 0·1 ml. of [$^{14}C_2$]glycine (2 μc/μmole), 427 000 counts/min. of ^{14}C; 10 μmoles of sodium formate (0·1 ml.) or 0·1 ml. of water as indicated. Total volume 1·2 ml. Tissues were killed and extracted as described in the text.

Fraction	[$^{14}C_2$]Glycine		[$^{14}C_2$]Glycine and formate	
	Counts/min.	% of ^{14}C incorporated	Counts/min.	% of ^{14}C incorporated
Lipid	6 300	1·5	5 600	1·5
Amino acids				
Neutral and basic amino acids, serine	90 740	21	2 600	0·6
Acidic amino acids	25 000	6	22 800	6
Sugars	1 900	0·4	3 600	0·9
Organic acids	27 000	6	49 400	13
Carbon dioxide	117 750	27	116 200	31
Residue	155 250	36	178 000	47
Total ^{14}C incorporated	426 540	—	378 200	—
% of [$^{14}C_2$]glycine utilized	99	—	88	—

Table 3. *Distribution of* ^{14}C *from* [$^{14}C_2$]*glycine in the organic acids of carrot tissues*

Cortical tissues (1 g.) of carrot roots were incubated for 6 hr. at 30° in Warburg flasks containing: 200 μmoles of tris buffer, pH 7·2; 0·1 ml. of [$^{14}C_2$]glycine (2 μc/μmole), 427 000 counts/min. of ^{14}C. Additions of 10 μmoles of sodium formate, 100 μmoles of sodium iodoacetate and sodium malonate were made as indicated. Total volume 1·2 ml. Organic acids were separated by the method of Palmer (1955).

Organic acid	[^{14}C]Glycine		[^{14}C]Glycine + formate		[^{14}C]Glycine + iodoacetate		[^{14}C]Glycine + malonate	
	Counts/min.	% of ^{14}C in organic acids	Counts/min.	% of ^{14}C in organic acids	Counts/min.	% of ^{14}C in organic acids	Counts/min.	% of ^{14}C in organic acids
Glyoxylate	10 200	38	11 000	22	7 300	33	2 900	54
Glycollate	Not active	—	3 100	6	Not active	—	1 700	32
Succinate	2 500	9	4 500	9	2 200	10	700	13
Malate	5 500	20	21 700	43	1 600	7	Not active	—
Citrate	2 300	8	2 000	4	Not active	—	Not active	—
Pyruvate fraction	2 500	9	4 200	8	8 200	37	Not active	—
Others	4 000	15	2 900	6	2 700	12	Not active	—
Total ^{14}C of organic acid fraction	27 000	—	49 400	—	22 000	—	5 300	—

solutions was increased to 90 min., the percentage of the total ^{14}C incorporated into the organic acids decreased. This was accompanied by increases in the percentages of ^{14}C found in the insoluble residue and in the carbon dioxide evolved during the experiments.

Effect of formate on glycine metabolism in carrot tissue. The effects of additions of formate to carrot tissues metabolizing glycine are shown in Table 2. In the presence of 10 μmoles of formate the amounts of ^{14}C recovered in the fractions isolated were reduced to 88 %. Furthermore, the soluble amino acids contained only small amounts of [^{14}C]serine when formate was supplied. Additions of formate were, however, clearly associated with increased amounts of ^{14}C from glycine being incorporated into the insoluble residue and into the organic acid fraction. Hydrolysis of the insoluble material indicated that the major radioactive component was [^{14}C]serine. Hence additions of formate, although not leading to large amounts of [^{14}C]serine in the free amino acid fraction, were associated with an increase in the incorporation of [$^{14}C_2$]glycine into protein serine.

The effect of additions of formate on the distribution of radioactivity in the organic acid fraction is shown in Table 3. When formate was added to the slices, greater amounts of [$^{14}C_2$]glycine were converted into malic acid. The radioactivity present in glyoxylate was not appreciably altered by this treatment, but glycollate was now detected and the radioactivity of succinate almost doubled.

Effects of iodoacetate and malonate on the metabolism of [$^{14}C_2$]glycine by carrot tissues. In studies on the effects of inhibitors on the metabolism of [$^{14}C_2$]-glycine, carrot tissue slices were incubated with the inhibitor for 30 min. before additions of the [$^{14}C_2$]-glycine. As is evident from Table 5, the presence of 100 μmoles of iodoacetate and 100 μmoles of malonate greatly reduced the amounts of [$^{14}C_2$]-glycine metabolism. In the control slices 99 % of the glycine was metabolized; this was decreased to 38 % and 1·9 % in the presence of iodoacetate and malonate respectively.

Inhibition by iodoacetate and malonate affected the radioactivity of all the fractions isolated. Furthermore, in the presence of 100 μmoles of iodoacetate, the distribution of ^{14}C in the various fractions was strikingly altered from that observed in the control slices. In the control tissues, 27 % of the incorporated [$^{14}C_2$]glycine was evolved as carbon dioxide; in the iodoacetate-treated slices this figure

Table 4. *Sequence of incorporation of ^{14}C into products of [$^{14}C_2$]glycine metabolism in carrot tissue slices*

Cortical tissues (1 g.) of carrot roots were incubated at 30° for the times indicated. Slices were incubated in 200 μmoles of tris buffer, pH 7·2, and 0·1 ml. of [$^{14}C_2$]glycine (2 μc/μmole), 427 000 counts/min. of ^{14}C. Total volume 1·1 ml.

Time of incubation (min.) ...	5		30		90	
Fraction	Counts/min.	% of ^{14}C incorporated	Counts/min.	% of ^{14}C incorporated	Counts/min.	% of ^{14}C incorporated
Lipid	200	7	500	6	600	3
Sugars	300	10	300	4	900	5
Organic acids	1 800	63	4 000	49	6 300	32
Carbon dioxide	60	3	500	6	1 200	6
Residue	500	17	2 900	35	10 500	54
Total ^{14}C incorporated	2 860	—	8 200	—	19 500	—
% of [$^{14}C_2$]glycine utilized	0·67	—	1·9	—	4·5	—

Table 5. *Effects of iodoacetate and malonate on the metabolism of [$^{14}C_2$]glycine by carrot tissue slices*

Cortical tissues (1 g.) of carrot root were incubated for 6 hr. at 30° as described in Table 3.

	[$^{14}C_2$]Glycine		[$^{14}C_2$]Glycine + iodoacetate		[$^{14}C_2$]Glycine + malonate	
Fraction	Counts/min.	% of ^{14}C incorporated	Counts/min.	% of ^{14}C incorporated	Counts/min.	% of ^{14}C incorporated
Lipid	6 300	1·5	3 200	2	300	4
Amino acids						
Neutral and basic amino acids, serine	90 740	21	Not active	—	Not active	—
Acidic amino acids	25 000	6	Not active	—	Not active	—
Sugars	1 900	0·4	1 200	0·7	500	6
Organic acids	27 000	6	22 000	13	5 300	64
Carbon dioxide	117 750	27	76 900	47	2 100	25
Residue	155 250	36	60 400	36	Not active	—
Total ^{14}C incorporated	426 540	—	163 700	—	8 200	—
% of [$^{14}C_2$]glycine utilized	99	—	38	—	1·9	—

Table 6. *Intramolecular distribution of* ^{14}C *in glycine and serine after feeding with* [$^{14}C_2$]*glycine*

Samples (10 000 counts/min.) of labelled glycine used in the feeding experiments or recovered from tissues after feeding for 6 hr. at 30° (see the Materials and Methods section) were mixed with 10 μmoles of carrier glycine and decarboxylated with ninhydrin. Activity in C-2 position is calculated by difference between counts/min. of sample and counts/min. recovered after ninhydrin treatment. Samples of [^{14}C]serine recovered from tissues after 6 hr. of [$^{14}C_2$]glycine metabolism were degraded with periodate (see the Materials and Methods section). Results are expressed as percentages of ^{14}C recovered from products of degradation procedures.

Amino acid	Carbon no.	Original feeding soln.	Extracted from carrot	Extracted from castor bean
Glycine	1	34	24	26
	2	66	76	74
Serine	1	—	17·8	21·1
	2	—	28·2	71·9
	3	—	54·0	7·0

was increased to 47 %. Similarly, the percentage of ^{14}C incorporated into the organic acids was increased from 6 % in the controls to 13 % in the iodoacetate-treated tissues. As is shown in Table 3, iodoacetate treatment resulted in large decreases in the activities recovered in glyoxylate and malate. In contrast, increases were observed in the ^{14}C content of the pyruvate fraction in the presence of iodoacetate.

Malonate, besides dramatically reducing the amounts of [$^{14}C_2$]glycine metabolized, greatly altered the distribution of ^{14}C in the fractions isolated. Radioactivity was not detected in the insoluble residue in the malonate-treated tissues but comprised 36 % of the ^{14}C incorporated in the controls (Table 5). Malonate treatment also affected the percentage of ^{14}C in the organic acid fraction: 6 % in the control, 64 % in the inhibited slices. The effect of malonate on the organic acids was also evident when this fraction was further separated (Table 3). Besides greatly reducing the amounts of radioactivity in the organic acids, malonate treatment resulted in ^{14}C being present in only glyoxylate, glycollate and in succinate. Radioactivity was not detected in malate, citrate or in the pyruvate fraction when malonate was present.

Intramolecular distribution of ^{14}C *in glycine and serine.* Table 6 shows the percentage distribution of ^{14}C in the glycine solutions fed to the tissues and the distribution found in samples of glycine extracted from the tissues after 6 hr. metabolism of [$^{14}C_2$]-glycine. The glycine fed to the tissues was found to have 66 % of ^{14}C in the C-2 position and 34 % in the C-1 position. After incubation with the tissues, this intramolecular distribution of ^{14}C was greatly altered. In both cases, greater percentages of ^{14}C were found in the C-2 position of the glycine molecule.

Degradation of the [^{14}C]serine produced from the carrot tissues metabolizing [$^{14}C_2$]glycine (Table 6) indicated that over 50 % of the ^{14}C content of the molecule was located in the C-3 position. In contrast, the C-3 position of serine produced by castor-bean endosperm contained only 7 % of the ^{14}C content of the serine molecule.

DISCUSSION

The formation of serine from glycine requires a C_1 compound which yields the C-3 atom of serine by a condensation involving tetrahydrofolate (Sakami, 1955). Such a reaction is reversible and has been demonstrated to occur in animal tissues (Kisliuk & Sakami, 1955) and in certain plant tissues (Wilkinson & Davies, 1960).

The C_1 compound required for serine biosynthesis in plants can be readily formed from formate (Tolbert, 1955; McConnell & Bilinski, 1959; E. A. Cossins & S. K. Sinha, unpublished work), from methanol (Cossins & Beevers, 1962) or from the C-2 position of glycollate (Tolbert & Cohan, 1953).

In the present experiments the C-3 position of serine was readily formed from [$^{14}C_2$]glycine by the carrot tissues (Table 6). Over 50 % of the ^{14}C present in the serine isolated was located in the C-3 position. In the castor-bean tissues only 7 % of the ^{14}C present in the isolated serine was in the C-3 position. It appears therefore that glycine, possibly via oxidation to glyoxylate, was readily split into a C_1 fragment which can give rise to the C-3 position of serine, the C-1 and C-2 positions of serine being derived from the corresponding positions of the glycine molecule. Clearly, if such a pathway were operating in the carrot tissues, considerably more glycine molecules would have to be split to yield the C-3 position of serine than were incorporated into the C-1 and C-2 positions directly.

In the castor-bean endosperm only small amounts of radioactivity were present in the C-3 position of serine (Table 6). This might be due to a considerable dilution of the C_1 fragment derived from glycine in a relatively large endogenous 'formate' pool. Glyoxylate is readily produced in this tissue from isocitrate (Carpenter & Beevers, 1959).

Oxidative decarboxylation of a portion of this endogenous glyoxylate might therefore reduce the amounts of ^{14}C entering the C-3 position of serine.

The production of formate and carbon dioxide from glycollate has been demonstrated in extracts of higher-plant tissues (Tolbert, Clagett & Burris, 1949). The reaction leading to cleavage of the molecule involved the intermediary formation of glyoxylate and was inhibited by iodoacetate and malonate. Similarly, in rat-liver slices glyoxylate is oxidatively decarboxylated to formate and carbon dioxide (Nakada, Friedmann & Weinhouse, 1955; Nakada & Sund, 1958).

In agreement with the postulated splitting of glyoxylate by a system similar to that reported by Tolbert *et al.* (1949), malonate and iodoacetate drastically reduced the amount of glycine metabolism in the present experiments (Table 5). Further, after malonate treatment glyoxylate contained a greater percentage of ^{14}C in the organic acid extract of the inhibited slices than did that from the control (Table 3). Also, labelled serine was not detected in these experiments with inhibitors.

When formate was added to slices of carrot tissues in addition to the micromolar amounts of [$^{14}C_2$]-glycine, the total amount of ^{14}C incorporated was reduced from 99 % in the control to 88 % where formate additions were made (Table 2). This treatment was accompanied by a large decrease in the amounts of [^{14}C]serine produced. When 1 μmole of labelled glycine was fed, over 90 000 counts/min. were recovered in serine. When, however, the 1 μ-mole of labelled glycine was accompanied by 10 μ-moles of unlabelled formate only 2600 counts/min. were detected in serine. Although the ^{14}C content of this latter serine was too low to allow degradations to be carried out, it is likely that this large pool of unlabelled formate greatly reduced the amounts of ^{14}C entering the C-3 position. In experiments with [^{14}C]formate of high specific activity (E. A. Cossins & S. K. Sinha, unpublished work) it has been demonstrated that serine, heavily labelled in the C-3 position, is readily produced by these tissues. The results are therefore consistent with a conversion of glycine via glyoxylate into a C_1 compound which might be formate or a compound that the tissues can readily synthesize from formate.

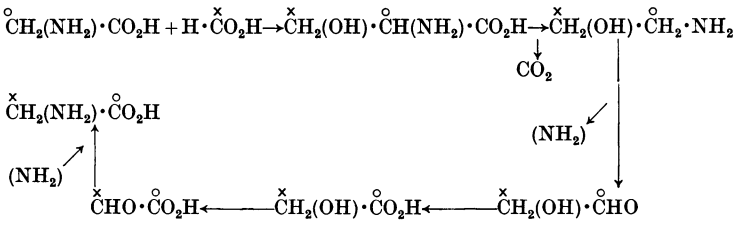
In addition to serine biosynthesis, glycine metabolism in the carrot and castor-bean tissues involved labelling of the organic acids (Tables 1 and 3). In both tissues the most heavily labelled organic acid was glyoxylate. Recently extracts have been prepared from carrot tissues (S. K. Sinha & E. A. Cossins, unpublished work) which produce glyoxylate from glycine by a transamination reaction. These preparations produce [^{14}C]glyoxylate when supplied with [^{14}C]glycine and pyruvate. The reaction is freely reversible and has been detected in a variety of plant tissues. Hence glycine metabolism in the castor-bean and carrot tissues probably involves a transamination reaction leading to production of glyoxylate.

In addition to labelling in glyoxylate, the castor-bean slices incorporated small amounts of ^{14}C into glycollate, malate and succinate. Labelling of malate and succinate could readily occur from [^{14}C]glyoxylate by a pathway of reactions involving malate synthetase, fumarase and isocitritase (Yamamoto & Beevers, 1960, 1961; Carpenter & Beevers, 1959; Canvin & Beevers, 1961; Beevers, 1961*a*). However, owing to the very low percentages of ^{14}C incorporated into the organic acids, other than glyoxylate, it appears that the utilization of glyoxylate derived from glycine does not involve organic acid metabolism to any great extent. Further, this glyoxylate was not extensively converted into carbohydrate.

In the present investigation glycine metabolism by slices of castor-bean endosperm involves mainly the production of serine, carbon dioxide and acidic amino acids. The small amounts of radioactivity in the organic acids isolated might therefore be due in part at least to the reactions of glyoxylate formation from glycine occurring at a different locality within the cell from the isocitritase reaction. Localization of certain metabolic reactions in the castor-bean endosperm tissues has been demonstrated by Tanner & Beevers (1963).

Labelling of malate, succinate, citrate and the pyruvate fraction was observed in the carrot tissues after periods of [$^{14}C_2$]glycine metabolism (Table 3). However, as there is considerable evidence that the enzymes of the glyoxylate cycle are mainly restricted to tissues metabolizing fat (Beevers, 1961*a*, *b*), the labelling of the organic acids in the carrot tissue is probably not the result of this cycle. Alternatively, labelling of the organic acids could occur by fixation of $^{14}CO_2$ into malate. Such carboxylation reactions leading to malate formation are of fairly widespread occurrence in higher plant tissues (Walker, 1962). Finally pyruvate or hydroxypyruvate might arise from [^{14}C]serine by a transamination reaction (Willis & Sallach, 1963). These pathways would conceivably yield products containing ^{14}C derived originally from glycine. By operation of the tricarboxylic acid cycle, the label might be further distributed among the organic acids isolated.

In both the tissues examined, the intramolecular distribution of ^{14}C in the glycine molecule was greatly altered during the experimental period (Table 6). Labelled glycine extracted from the tissues after incubation for 6 hr. at 30° contained a higher percentage of ^{14}C in the C-2 position than was observed at the start of the experiment. It appears therefore that considerable breakdown and



Scheme 1

resynthesis of the glycine molecule occurred during the experiments.

Resynthesis of glycine via ethanolamine might possibly account for the increased ^{14}C content of the C-2 position of glycine extracted from the carrot tissues. There is evidence from studies with animal tissues that glycine can be converted into ethanolamine *in vivo* by way of the intermediary formation of serine, which is decarboxylated (Elwyn, Weissbach, Henry & Sprinson, 1955). The ethanolamine so formed can then be converted into glycine via glycolaldehyde, glycollate and glyoxylate (Fruton & Simmonds, 1958). Operation of this 'glycine–ethanolamine cycle' (Scheme 1) thus leads to the conversion of the C-3 position of serine into the C-2 position of glycine. Thus in carrot tissues, where the C-3 position of serine contains 54 % of the ^{14}C content of the serine molecule (Table 6), these reactions, if operating according to Scheme 1, might lead to production of glycine with a greater percentage of ^{14}C in the C-2 position.

In the castor-bean endosperm, however, the enrichment of the C-2 position of glycine during the experiments cannot be explained on the basis of a glycine–ethanolamine cycle. In these tissues, the C-3 position of serine contained only 7 % of the ^{14}C content of the molecule (Table 6). If this serine were involved in the reactions shown in Scheme 1, glycine more heavily labelled in the carboxyl carbon atom would be produced. Thus the changes in the intramolecular distribution of ^{14}C in glycine extracted from the castor-bean tissues cannot be readily explained on the basis of established pathways for glycine metabolism and synthesis.

SUMMARY

1. The metabolism of glycine in storage tissues of carrot and the endosperm of 5-day-old castor-bean seedlings has been studied with micromolar amounts of [$^{14}C_2$]glycine.

2. The main products of 6 hr. [$^{14}C_2$]glycine metabolism in both tissues were serine, glyoxylate and carbon dioxide.

3. Additions of malonate and iodoacetate to the carrot tissues greatly reduced the amounts of ^{14}C entering the fractions isolated. Both inhibitors decreased the amounts of ^{14}C detected in serine, and malonate increased the percentage of the label in glyoxylate.

4. Degradations of [^{14}C]serine produced from [$^{14}C_2$]glycine feeding indicated that, besides contributing to the C-1 and C-2 positions of serine, labelling of the C-3 position had occurred.

5. Degradations of [$^{14}C_2$]glycine remaining in the tissues after the experimental periods indicated that considerable breakdown and resynthesis of glycine had occurred.

6. The results are interpreted as being consistent with a conversion of glycine into glyoxylate, possibly via a transamination reaction. Glyoxylate, possibly by oxidative decarboxylation, contributes to the C-3 position of serine. Changes in the intramolecular distribution of ^{14}C in glycine might indicate breakdown and resynthesis of glycine in the carrot tissues by a pathway established for animal tissues.

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Comparative Studies of 'Bile Salts'

20. BILE SALTS OF THE COELACANTH, *LATIMERIA CHALUMNAE* SMITH*

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A preliminary examination of *Latimeria* bile salts showed that they were mainly of the alcohol sulphate type, that a mixture of at least three substances was present and that the principal bile alcohol gave an infrared-absorption spectrum indicating that it had a steroid nucleus different from that present in cholic acid or in the bile alcohols ranol and cyprinol (Haslewood, 1957). Because of the great rarity of the bile, obtained in the present instance from a coelacanth kept alive for some hours in captivity (Millot, 1955), we decided to postpone further work on it until techniques for the examination of bile alcohols had been improved and the chemistry of these substances was better understood.

We now report a detailed examination of *Latimeria* bile salts.

RESULTS

Cleavage of the dried bile salts with the dioxan–trichloroacetic reagent gave as the principal product an easily purified alcohol that we call 'latimerol'. Latimerol, m.p. 236°, $[\alpha]_D + 33°$, had an infrared-absorption spectrum (Fig. 1*a*) that was indeed different from those of substances containing either the cholic acid or the allocholic acid nucleus. However, the latimerol spectrum did show some of the bands (especially those at about 10·4 and 11·2 μ) that we have come to associate with the nucleus of allocholic acid (Anderson, Briggs & Haslewood, 1964), and, since latimerol gave a precipitate with digitonin solutions and also a purple colour in the Hammersten test, we thought that it might have the substituted ring structure of the unknown 3β,7α,12α-trihydroxyallocholanic acid. Attempts to make this acid from allocholic acid [3α,7α,12α-trihydroxyallo(5α)cholanic acid] and from other likely starting materials all failed, and we finally resorted to a method used by Danielsson, Kallner & Sjövall (1963) for the preparation of allodeoxycholic acids. This method [modified in turn from a procedure outlined by Chakravati, Chakravati & Mitra (1962)] involved in our case the partial epimerization (at C-5) of ethyl 7α,12α-dihydroxy-3-oxocholanate (I) by prolonged heating with Raney nickel in cumene. The resulting mixture

* Part 19: Haslewood (1964).