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The Interconversion of Glycine and Serine by Plant Tissue Extracts

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1. Extracts prepared from a variety of higher-plant tissues by ammonium sulphate fractionation were shown to catalyse the interconversion of glycine and serine. This interconversion had an absolute requirement for tetrahydrofolate and appeared to favour serine formation. 2. The biosynthesis of serine from glycine was studied in more detail with protein fractionated from 15-day-old wheat leaves. Synthesis of [^{14}C]serine from [^{14}C]glycine was not accompanied by labelling of glyoxylate, glycollate or formate. 3. The synthesis of serine from glycine was stimulated by additions of formaldehyde, and [^{14}C]formaldehyde was readily incorporated into C-3 of serine in the presence of tetrahydrofolate. 4. The results are interpreted as indicating that serine biosynthesis involves a direct cleavage of glycine whereby the α-carbon is transferred via N^5N^{10}-methylenetetrahydrofolate to become the β-carbon of serine.

Early studies with animal tissues established that serine could be rapidly converted into glycine with loss of the β-carbon as a C_1 unit (Sakami, 1955; Siekevitz & Greenberg, 1949, 1950; Shemin, 1946). Since these reports, a large number of investigators have studied the enzymic interconversion of glycine and serine, mainly with extracts prepared from a large variety of animal tissues (Sakami, 1955; Huennekens & Osborn, 1959; Rabinowitz, 1960). These studies stress the importance of this interconversion in amino acid metabolism and have also demonstrated the role of tetrahydrofolate in C_1 transfer.

The interconversion of glycine and serine in plants has been demonstrated by several workers (McConnell & Bilinski, 1959; Wang & Waygood, 1962; Wang & Burris, 1963; Wilkinson & Davies, 1958; Sinha & Cossins, 1964). Most of these studies were carried out with intact plant tissues and involved the administration of small quantities of [^{14}C]glycine and [^{14}C]serine. However, Wilkinson & Davies (1960) have shown that extracts of some plant tissues catalyse the interconversion of glycine and serine by a mechanism similar to that described for animal tissues.

There is now considerable evidence that the interconversion of glycine and serine is catalysed by the enzyme serine hydroxymethyltransferase (EC 2.1.2.1) (eqn. 1). This enzyme has been

$$\text{Serine} + \text{tetrahydrofolate} \rightleftharpoons \text{glycine} + N^5N^{10}\text{-methylenetetrahydrofolate} \quad (1)$$

extensively studied with mammalian and avian tissues (Sakami, 1955; Blakley, 1955; Hatefi, Osborn, Kay & Huennekens, 1957; Alexander & Greenberg, 1955, 1956; Schirch & Mason, 1952) and with bacterial preparations (Lascelles & Woods, 1954; Wright, 1955). It is also present in some plant tissues (Wilkinson & Davies, 1960).

Serine hydroxymethyltransferase catalyses a freely reversible reaction that can result in the formation of N^5N^{10}-methylenetetrahydrofolate, a compound playing a central role in C_1 metabolism (Huennekens & Osborn, 1959; Friedkin, 1963). Alternatively, this reaction can result in the biosynthesis of serine from glycine and N^5N^{10}-methylenetetrahydrofolate. There is considerable evidence that N^5N^{10}-methylenetetrahydrofolate can arise enzymically from formate, methanol and formaldehyde (Sakami, 1955; Osborn, Vercamer, Tolbert & Huennekens, 1957) or spontaneously by reaction of excess of formaldehyde with tetrahydrofolate (Kisliuk, 1957).

Studies of serine biosynthesis in plants have suggested that serine hydroxymethyltransferase plays a major role. During short periods of photosynthesis with $^{14}CO_2$, this amino acid is commonly more heavily labelled in the β-carbon than in the carboxyl carbon (Rabson, Tolbert & Kearney, 1962). This suggests that serine biosynthesis under these conditions involves a heavily labelled C_1 unit that condenses with glycine rather than transamination of hydroxypyruvate arising from labelled 3-phosphoglyceric acid. The C_1 units required in this biosynthesis could conceivably arise from glycollate, which is commonly heavily labelled during photosynthesis in $^{14}CO_2$ (Zelitch, 1964).

Some plant tissues contain a glycollate pathway

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whereby glycollate can be utilized for sugar and amino acid biosynthesis (Rabson *et al.* 1962; Wang & Waygood, 1962; Jiminez, Baldwin, Tolbert & Wood, 1962), e.g.: glycollate → glyoxylate → glycine → serine → hydroxypyruvate → sugars. Enzymes catalysing the partial reactions of this sequence are particularly active in chlorophyll-containing tissues (Zelitch, 1964; Willis & Sallach, 1963; Cossins & Sinha, 1965*a*). However, no data are available on serine-hydroxymethyltransferase activity in green plants.

In the present studies, extracts have been prepared from a variety of plant tissues that are known to be active in serine biosynthesis (Cossins, 1964; Cossins & Sinha, 1964, 1965*b*; Sinha & Cossins, 1965). These extracts were examined for ability to synthesize C_1 units from the α-carbon of glycine and to utilize these in the serine-hydroxymethyltransferase reaction. Glycine and serine were found to be freely interconvertible in the presence of tetrahydrofolate. However, serine formation was favoured, particularly when micromolar quantities of formaldehyde were present. A preliminary report of this work has already appeared (Cossins & Sinha, 1965*c*).

MATERIALS AND METHODS

Plant materials. Seeds of pea (*Pisum sativum* L. var. Homesteader), corn (*Zea mays* L. var. Falconer), wheat (*Triticum sativum*) and castor bean (*Ricinus communis* L. var. *zanzibarensis*) were soaked overnight in water at 25°. After this soaking period the seeds were transferred to moist vermiculite and allowed to germinate at 30°. Germination of corn and castor-bean seeds was carried out in the dark for 5 days. In experiments with pea and wheat leaves, the seeds were germinated in a 12hr. light and 12hr. dark cycle at 30° for 15 days. The storage tissues of carrot (*Daucus carota* L.) were purchased locally and stored at 5° before preparation of the extracts.

Chemicals and labelled substrates. DL-Tetrahydrofolic acid in M-2-mercaptoethanol and L-glycine were purchased from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). [2-^{14}C]Glycine, [1-^{14}C]glycine and [1-^{14}C]serine were purchased from California Corp. for Biochemical Research (Los Angeles, Calif., U.S.A.) and were diluted with carrier glycine and serine to give solutions with final specific activities of 3 μc/μmole/0·1 ml. of solution. [^{14}C]-Formaldehyde was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.) and diluted with water to give a final specific activity of 1·5 μc/μmole/0·1 ml. of solution.

Preparation and fractionation of plant extracts. Extracts of the plant tissues were prepared by grinding samples (50g. fresh wt.) with 150 ml. portions of 0·1 M-phosphate buffer, pH 7·6, at 2° in a hand-operated blender. The homogenates were then passed through fine muslin and centrifuged at 10000*g* for 20 min. in an International refrigerated centrifuge (Servall) operated at 2°. To the supernatants, quantities of ice-cold saturated $(NH_4)_2SO_4$ were added until the final concentration was 80% saturation. In some cases, saturated $(NH_4)_2SO_4$ was added until 33%, 50% and 75% saturation were reached. After standing for 20 min. at 2°, the precipitated protein was collected by centrifugation at 10000*g* for 10 min. at 2° and dissolved in a small volume (approx. 20 ml.) of 0·1 M-phosphate buffer, pH 7·6. The protein solutions were then dialysed against 1l. of 0·1 M-phosphate buffer, pH 7·6, for 20hr. at 2°.

Assay of amino acid biosynthesis. Incubation of the protein extracts with labelled substrates was carried out at 30° in conventional Warburg flasks. The side arm of each flask contained the following components in a total volume of 0·3 ml.: DL-tetrahydrofolate, 10 μmoles; labelled substrate, 1 μmole containing 2–3 μc of ^{14}C; 2-mercaptoethanol, 20 μmoles. The main compartment of each flask initially contained 2 ml. of the plant extract in 0·1 M-phosphate buffer, pH 7·6. The flasks were connected to Warburg manometers and equilibrated for 10 min. at 30°. The components of the reaction system were then mixed and the incubation was continued for up to 2hr. In experiments where the labelled substrate was [1-^{14}C]glycine, the procedure was identical except that 0·2 ml. of 20% (w/v) carbonate-free NaOH solution was placed in the centre well to absorb any $^{14}CO_2$ evolved during the incubation.

Analytical methods. At the end of the experimental periods, the reaction was terminated by adding 10 ml. of 80% (v/v) ethanol at −10°. The precipitated protein was removed by centrifugation, washed with 5 ml. of ice-cold water and centrifuged again. The combined supernatants from these centrifugations were concentrated to 2 ml. *in vacuo* at 40° and passed through columns (1 cm. × 6 cm.) of Dowex 1 (X10; H^+ form). The amino acids were eluted from the columns by adding 40 ml. of 2 N-NH_3 solution. The effluent was then concentrated *in vacuo* at 40° and the radioactive components were separated by descending paper chromatography. Preliminary experiments demonstrated that the principal labelled products were N^5N^{10}[^{14}C]-methylenetetrahydrofolate, [$^{14}C_2$]glycine and [$^{14}C_3$]serine. To separate these radioactive compounds, one-dimensional paper chromatography was employed with butan-1-ol–acetone–water–diethylamine (20:20:10:3, by vol.) as solvent.

Radioactive areas on the paper chromatograms were detected by scanning with a Nuclear–Chicago 4Pi Actigraph (model 4502). The radioactive areas were then eluted from the paper chromatograms with water, and portions of the eluate (0·2 ml.) were plated on to nickel-plated steel planchets and dried under an infrared lamp. Radioactivity was then assayed in a gas-flow counter with 10% efficiency (model C110B; Nuclear–Chicago Corp., Chicago, Ill., U.S.A.). The counts were corrected for background. In experiments where CO_2 was collected during the reaction period, the contents of the centre well were mixed with 5 ml. of 10% (w/v) $BaCl_2$ solution, and the $BaCO_3$ so formed was filtered off on glass-fibre filter-paper disks. The disks were dried thoroughly at 100°, mounted on nickel-plated planchets and assayed for radioactivity in the gas-flow counter. The counts were corrected for self-absorption and background.

Protein content of the plant extracts was determined spectrophotometrically by the method of Warburg & Christian (1941).

Degradation of labelled amino acids. (*a*) Glycine. Samples of [^{14}C]glycine formed after incubating plant extracts with labelled serine were degraded with 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, ^{14}C recovered from the carboxyl group contained more than 90% of the total radioactivity of the sample before ninhydrin treatment.

(*b*) Serine. Samples of labelled serine recovered from the plant extracts as described above were degraded with periodate (Sakami, 1950). The products of periodate oxidation were recovered as [^{14}C]formaldehyde dinitrophenylhydrazone, sodium [^{14}C]formate and $Ba^{14}CO_3$ (Sinha & Cossins, 1964). These labelled compounds were plated on to nickel-plated planchets and assayed for radioactivity as described by Sinha & Cossins (1964).

RESULTS

Interconversion of glycine and serine by plant tissue extracts. In preliminary experiments it was observed that serine and glycine were only interconvertible in the presence of tetrahydrofolate. In a survey of various plant tissues for ability to catalyse this interconversion, ammonium sulphate-fractionated extracts that had been dialysed were used (Tables 1 and 2). It is clear that considerable amounts of radioactivity were incorporated into serine when [2-^{14}C]glycine was supplied to all of the tissue extracts (Table 1). Although the specific radioactivities of the labelled glycine and serine supplied in these experiments were equal, the interconversion of these amino acids appears to favour serine formation. Degradation of the serine formed from [2-^{14}C]glycine showed that it was labelled entirely in C-2 and C-3. Degradation of the glycine formed from [1-^{14}C]serine showed that it was labelled only in C-1. During these interconversions only very small amounts (less than 100 counts/min.) of $^{14}CO_2$ were evolved. In further experiments with wheat-leaf extracts with [1-^{14}C]-glycine (6×10^5 counts/min.), under the same experimental conditions as in Table 1, approx. 1000 counts/min. were detected in the carbon dioxide evolved during the 2hr. experimental period. In this latter experiment, the [^{14}C]serine formed (18000 counts/min.) was labelled entirely in C-1 (Table 3). In no case was labelled glyoxylate, glycollate or formate detected during the interconversion of glycine and serine.

The formation of serine from glycine catalysed by wheat-leaf extracts was examined in more detail in further experiments. For example, this biosynthesis was markedly affected by pH (Fig. 1). Maximal serine formation occurred when the reaction took place at pH 8·0.

In a further experiment with wheat-leaf extracts

Table 1. *Synthesis of serine from glycine by extracts of higher-plant tissues*

Each reaction flask contained in a total volume of 2·3ml.: 2ml. of enzyme preparation [0–80%-saturated $(NH_4)_2SO_4$ fraction]; 10μmoles of tetrahydrofolate; 1μmole of [2-^{14}C]glycine (6×10^5 counts/min.); 20μmoles of mercaptoethanol; 200μmoles of phosphate buffer, pH 7·2. The mixture was incubated at 30° in air for 2hr.

	Protein (mg./ml. of extract)	[^{14}C]Serine synthesized (counts/min.)	Specific enzyme activity (counts/min. in [^{14}C]serine/mg. of protein/hr.)
Carrot storage tissue	9·5	33450	3520
Castor-bean 5-day-old endosperm	25·0	64500	2580
Corn coleoptiles	13·0	71100	5470
Pea leaves	33·0	28000	850
Wheat leaves	18·0	37000	2050

Table 2. *Synthesis of glycine from serine by extracts of higher-plant tissues*

Each reaction flask contained in a total volume of 2·3ml.: 2ml. of enzyme preparation [0–80%-saturated $(NH_4)_2SO_4$ fraction]; 10μmoles of tetrahydrofolate; 1μmole of [1-^{14}C]serine (6×10^5 counts/min.); 20μmoles of mercaptoethanol; 200μmoles of phosphate buffer, pH 7·2. The mixture was incubated at 30° in air for 2hr.

	Protein (mg./ml. of extract)	[^{14}C]Glycine synthesized (counts/min.)	Specific enzyme activity (counts/min. in [^{14}C]glycine/mg. of protein/hr.)
Castor-bean 5-day-old endosperm	25·0	35250	1410
Corn coleoptiles	13·0	14200	1092
Wheat leaves	18·0	3220	179

Table 3. *Intramolecular distribution of* ^{14}C *in serine synthesized by wheat-leaf extracts*

Samples of [^{14}C]serine recovered after incubation of wheat-leaf extracts, as described in Tables 1, 5 and 7, were degraded with periodate. The results are expressed as percentages of the ^{14}C recovered from products of the degradation procedures. The results are averages of three separate determinations.

		Distribution of ^{14}C (%)			
	Carbon no.	[1-^{14}C]Glycine added	[2-^{14}C]Glycine added	[2-^{14}C]Glycine + formaldehyde added	[^{14}C]Formaldehyde + glycine added
CO_2H	1	100	0	0	0
$CH \cdot NH_2$	2	0	53·0	100	0
$CH_2 \cdot OH$	3	0	47·0	0	100

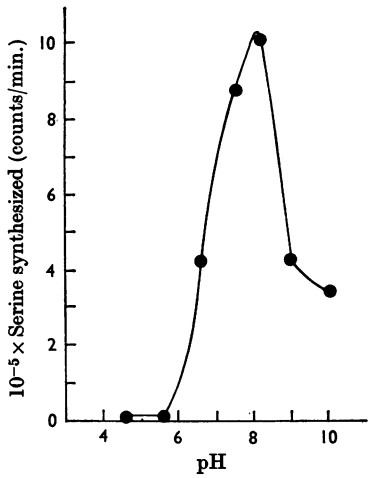

Fig. 1. Relationship between serine synthesis and pH. Samples of wheat-leaf extract [50–75%-saturated $(NH_4)_2SO_4$ fraction] containing 20mg. of protein were incubated with DL-tetrahydrofolate (10μmoles), [2-^{14}C]-glycine (1μmole containing 2μc of ^{14}C), 2-mercaptoethanol (20μmoles) and phosphate buffer (200μmoles), pH as indicated. The total volume was 3ml. After incubation for 1hr. at 30° the reaction was terminated by adding 80% (v/v) ethanol at −10°.

the initial crude homogenate was fractionated with ammonium sulphate (Table 4). Protein that was precipitated at 50–75% saturation with ammonium sulphate had the highest specific enzyme activity. The increase in specific activity that accompanied protein fractionation indicates a 92-fold purification of the enzyme system involved in serine biosynthesis from glycine. The fraction precipitated at 50–75% saturation with ammonium sulphate retained its ability to catalyse the biosynthesis of serine even after dialysis for 48hr. against 0·1M-phosphate buffer, pH7·6, and after storing at 2° for 2 weeks.

Table 4. *Fractionation of wheat-leaf extracts catalysing the biosynthesis of serine from glycine*

Enzyme activity was assayed by the amount of radioactivity in [^{14}C]serine synthesized after incubating leaf protein with 1μmole of [2-^{14}C]glycine (6×10^5 counts/min.), 10μmoles of tetrahydrofolate, 20μmoles of mercaptoethanol and 200μmoles of phosphate buffer, pH7·6, for 1hr. at 30°. The total volume was 3·0ml. One unit of enzyme activity is the amount of enzyme catalysing the production of 1000 counts/min. in [^{14}C]serine under the defined experimental conditions.

Fraction	Specific enzyme activity (units/mg. of protein)
Crude homogenate	1·34
0–33% saturated with $(NH_4)_2SO_4$	5·30
35–50% saturated with $(NH_4)_2SO_4$	19·33
50–75% saturated with $(NH_4)_2SO_4$	124·10

Role of N^5N^{10}*-methylenetetrahydrofolate in glycine–serine interconversion.* The incorporation of C-2 of glycine into C-3 of serine and the requirement for tetrahydrofolate strongly suggest that glycine can give rise to an activated C_1 unit which serves as a precursor of the C-3 of serine. If the conversion of glycine into serine involves a direct cleavage of the glycine molecule, it is possible that N^5N^{10}-methylenetetrahydrofolate would be produced (Sagers & Gunsalus, 1961). In experiments with wheat-leaf extracts, the conversion of glycine into serine only occurred in the presence of tetrahydrofolate (Fig. 2). If EDTA was also added, a third radioactive compound was detected (Fig. 2). This compound was not labelled if tetrahydrofolate was omitted during incubation with EDTA and it displayed marked fluorescence in ultraviolet light. The absorption spectrum of this compound and its mobility on paper chromatograms developed in butan-1-ol–acetone–water–diethylamine (20:20:10:3, by vol.) were identical with those given by N^5N^{10}-methylenetetrahydrofolate generated by

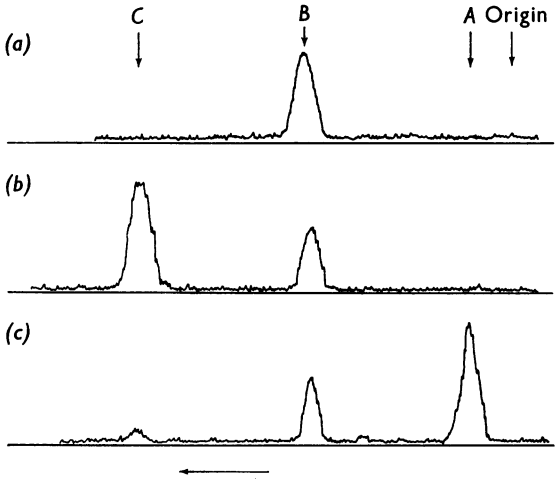

Fig. 2. Requirements for the biosynthesis of serine from glycine by wheat-leaf extracts. Actigraph tracings of paper chromatograms are shown. Paper chromatography was carried out with butan-1-ol–acetone–water–diethylamine (20:20:10:3, by vol.) in the direction shown by the horizontal arrow. Peaks: *A*, N^5N^{10}-methylenetetrahydrofolate; *B*, glycine; *C*, serine. (*a*) Leaf extract + [2-^{14}C]glycine; (*b*) leaf extract + [2-^{14}C]glycine + tetrahydrofolate; (*c*) leaf extract + [2-^{14}C]glycine + tetrahydrofolate + EDTA (0·01 M).

reaction of excess of formaldehyde and tetrahydrofolate. This third compound was therefore tentatively identified as N^5N^{10}-methylenetetrahydrofolate.

If N^5N^{10}-methylenetetrahydrofolate is formed by a direct cleavage of glycine, it is possible that a further molecule of glycine would be utilized to yield C-1 and C-2 of the serine produced. This suggestion is substantiated by the observed labelling pattern of the [^{14}C]serine produced from [2-^{14}C]glycine (Table 3). Further evidence in support of this suggestion might be provided from a study of the effects of various C_1 compounds on this conversion. For example, formaldehyde can give rise to N^5N^{10}-methylenetetrahydrofolate by reaction with tetrahydrofolate (Kisliuk, 1957). Methanol and formate would, however, only give rise to this tetrahydrofolate derivative indirectly (Friedkin, 1963). Clearly any compound giving rise to N^5N^{10}-methylenetetrahydrofolate would tend to increase the total incorporation of glycine carbon into serine and alter the incorporation into the C-3 of serine. Table 5 shows the results of a typical experiment in which methanol, formate and formaldehyde were added in addition to the usual reaction components. Methanol had no effect on the incorporation of labelled glycine into serine. However, formate and formaldehyde both led to

Table 5. *Effects of methanol, formate and formaldehyde on the conversion of glycine into serine by wheat-leaf extracts*

Reaction flasks contained in a total volume of 2·3 ml.: 2 ml. of enzyme preparation [50–75%-saturated $(NH_4)_2SO_4$ fraction; 20 mg. of protein/ml.]; 10 μmoles of tetrahydrofolate; 1 μmole of [2-^{14}C]glycine (6×10^5 counts/min.); 20 μmoles of mercaptoethanol; 200 μmoles of phosphate buffer, pH 7·2. Additions of methanol, formate and formaldehyde (10 μmoles) were made as indicated. The mixture was incubated at 30° in air for 1 hr.

Treatment	[^{14}C]Serine synthesized (counts/min.)
[2-^{14}C]Glycine	169000
[2-^{14}C]Glycine + methanol	171000
[2-^{14}C]Glycine + formate	217000
[2-^{14}C]Glycine + formaldehyde	385000

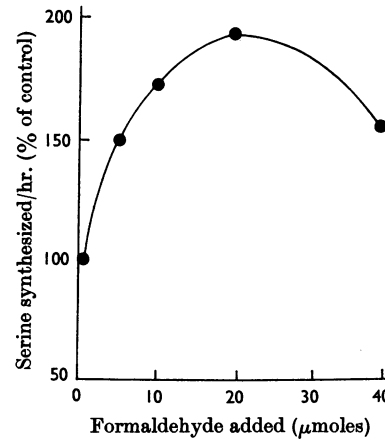

Fig. 3. Effect of formaldehyde on the biosynthesis of serine from glycine. Samples of wheat-leaf extract [50–75%-saturated $(NH_4)_2SO_4$ fraction] containing 20 mg. of protein were incubated as described in the Materials and Methods section in the presence of formaldehyde as indicated.

an increase in the amounts of labelled serine formed. In the presence of formaldehyde the serine formed contained more than twice the radioactivity of that formed in the presence of [2-^{14}C]glycine alone. Degradation of serine formed from [2-^{14}C]glycine in the presence of formaldehyde showed consistently that only C-2 was labelled (Table 3). The stimulatory effect of formaldehyde on the formation of serine from glycine was affected by the amounts of formaldehyde added (Fig. 3). As in the previous experiments, the control without formaldehyde produced large amounts of labelled serine from [2-^{14}C]glycine. However, this was again increased by adding small quantities of formaldehyde up to

20 μmoles. Above this, formaldehyde appears to be inhibitory.

Incorporation of [^{14}C]*formaldehyde into serine.* If additions of formaldehyde stimulate the conversion of glycine into serine because formaldehyde is actually incorporated into serine this should be demonstrable by using labelled formaldehyde. Wheat-leaf extracts readily synthesized serine from labelled formaldehyde and this ability was purified over 50-fold by fractionation with ammonium sulphate (Table 6). In all experiments with labelled formaldehyde, preincubation with tetrahydrofolate led to an extensive formation of [^{14}C]methylenetetrahydrofolate. When glycine was present in this system together with a wheat-leaf preparation, [^{14}C]serine was formed (Table 7). The synthesis of serine in this system occurred under aerobic or anaerobic conditions. Serine formed from [^{14}C]-formaldehyde was labelled in C-3 only (Table 3).

Table 6. *Fractionation of wheat-leaf extracts catalysing the biosynthesis of serine from formaldehyde*

Enzyme activity was assayed by the amount of radioactivity in [^{14}C]serine synthesized after incubating leaf protein with 1 μmole of [^{14}C]formaldehyde (3×10^5 counts/min.), 10 μmoles of glycine, 10 μmoles of tetrahydrofolate, 20 μmoles of mercaptoethanol and 200 μmoles of phosphate buffer, pH 7·6, for 1 hr. at 30°. The total volume was 3·0 ml. One unit of enzyme activity is the amount of enzyme catalysing the production of 1000 counts/min. in [^{14}C]serine under the defined experimental conditions.

Fraction	Specific enzyme activity (units/mg. of protein)
Crude homogenate	5·0
0–33% saturated with $(NH_4)_2SO_4$	12·5
33–50% saturated with $(NH_4)_2SO_4$	23·1
50–75% saturated with $(NH_4)_2SO_4$	278·1

Table 7. *Incorporation of* [^{14}C]*formaldehyde into serine by wheat-leaf extracts*

The complete reaction mixture contained in a total volume of 2·3 ml.: 2 ml. of enzyme preparation [50–75%-saturated $(NH_4)_2SO_4$ fraction; 20 mg. of protein/ml.]; 1 μmole of [^{14}C]formaldehyde (3×10^5 counts/min.); 10 μmoles of tetrahydrofolate; 20 μmoles of mercaptoethanol; 200 μmoles of phosphate buffer; 4 μmoles of glycine. The mixture was incubated at 30° for 1 hr.

Reaction system	[^{14}C]Serine synthesized (counts/min./hr.)
Complete (incubated in air)	94000
Complete (incubated in N_2)	95000
Minus glycine (incubated in air)	22000
Minus tetrahydrofolate (incubated in air)	200
Minus tetrahydrofolate and glycine (incubated in air)	0

DISCUSSION

The results of the present experiments have shown that C-2 of glycine can give rise to C-3 of serine *in vitro*. This conversion of glycine into serine had an absolute requirement for tetrahydrofolate and was stimulated by the addition of formaldehyde (Fig. 3). These findings strongly suggest that the extracts possessed the ability to catalyse the formation of activated C_1 units from the α-carbon of glycine.

Several investigators have shown that the α-carbon of glycine can give rise to C_1 units that may be utilized in various biosyntheses. For example, studies with animal tissues and with various bacteria have shown that C_1 units arising from the α-carbon of glycine can be utilized in (*a*) purine and thymine biosynthesis (Elwyn & Sprinson, 1950; Rege & Sreenivasan, 1954), (*b*) methionine biosynthesis (Stekol, 1955; Arnstein & Neuberger, 1953; Cross & Woods, 1954) and (*c*) serine biosynthesis (Sakami, 1955; Siekevitz & Greenberg, 1950; Arnstein & Neuberger, 1953; Winnick, Moring-Claesson & Greenberg, 1948).

There appear to be three main pathways whereby C_1 units can arise from the α-carbon of glycine. First, this may occur indirectly via δ-aminolaevulic acid (Huennekens & Osborn, 1959). Secondly, C_1 units may arise via glyoxylate produced from glycine by transamination. Glyoxylate can then be converted into formate and carbon dioxide by an oxidative cleavage (Nakada & Weinhouse, 1953; Tolbert, Clagett & Burris, 1949; Cossins & Sinha, 1965*b*). Formate is known to enter C_1 metabolism after being activated in the N^{10}-formyltetrahydrofolate-synthetase reaction (Friedkin, 1963). Thirdly, C_1 units are known to arise directly from the α-carbon of glycine by a reaction involving a direct cleavage and formation of N^5N^{10}-methylenetetrahydrofolate. Evidence for a direct cleavage of glycine has been obtained from studies with *Clostridium acidi-urici* and *Diplococcus glycinophilus* (Sagers & Gunsalus, 1961) and with *Escherichia coli* by Pitts & Crosbie (1962). Further, work reported by Richert, Amberg & Wilson (1962) clearly shows that similar reactions are catalysed by avian liver extracts.

In the present studies, the biosynthesis of serine from glycine was not accompanied by labelling of δ-aminolaevulate, glyoxylate or formate. Further, the possibility of an endogenous source of C_1 units in the extracts is unlikely as fractionation and dialysis of the preparations actually increased the specific enzyme activity (Table 4). Similarly it can be argued that the extensive incorporation of C-2 of glycine into C-3 of serine indicates that no endogenous C_1 source was available except that of the labelled glycine supplied.

The results of the present studies strongly suggest that glycine can be cleaved directly to yield N^5N^{10}-methylenetetrahydrofolate, which accumulates in the presence of EDTA (Fig. 2). Although detailed studies of the products of this reaction have not been made in the present work, it is conceivable that the reaction proceeds according to eqn. (2). The activated C_1 unit formed could

$$\text{Glycine} + \text{tetrahydrofolate} \rightarrow N^5N^{10}\text{-methylenetetrahydrofolate} + NH_3 + CO_2 \quad (2)$$

then be utilized in the serine-hydroxymethyltransferase reaction (eqn. 1), resulting in the formation of serine. In the overall reaction (eqn. 3), 2 mol. of glycine will be utilized in the formation of 1 mol. of serine. If this system is studied with

$$2\ \text{Glycine} \rightarrow \text{serine} + CO_2 + NH_3 \quad (3)$$

the aid of labelled glycine, [1-^{14}C]glycine will give rise to $^{14}CO_2$ and [1-^{14}C]serine. Experiments with [2-^{14}C]glycine should result in the formation of serine labelled at C-2 or C-3 or both and no significant amounts of $^{14}CO_2$ should be produced.

The present studies have shown that these predictions are in part fulfilled. For example, approximately twice as much radioactivity was detected in serine when [2-^{14}C]glycine was used as compared with experiments with [1-^{14}C]glycine. In all such experiments, the labelled solutions had equal specific radioactivities. Further, serine formed from [2-^{14}C]glycine was labelled in C-2 and C-3, and that formed from [1-^{14}C]glycine was labelled exclusively in C-1 (Table 3). Labelled carbon dioxide was produced in detectable amounts when [1-^{14}C]glycine was incubated with plant protein in the presence of tetrahydrofolate but only in trace amounts when [2-^{14}C]glycine was added under the same experimental conditions. In experiments with [1-^{14}C]glycine the radioactivity in serine was always greater than that detected in carbon dioxide. Clearly, if serine biosynthesis by wheat-leaf extracts involves a direct cleavage of glycine as shown by eqn. (3), the total radioactivity in carbon dioxide and serine should be equal. In no experiment was this demonstrated even if 0·1 ml. of 10% (w/v) trichloroacetic acid was used to terminate the reaction. Clearly further work with more highly purified preparations is necessary to determine the exact stoicheiometry of the reactions involved.

The ready incorporation of [^{14}C]formaldehyde into serine in the presence of glycine and tetrahydrofolate (Table 7) strongly suggests that serine hydroxymethyltransferase was present in the wheat extracts. Fractionation of the glycine→serine system and the formaldehyde→serine system yielded similar results (Tables 4 and 6), namely that both reactions are catalysed most readily by the fraction precipitated at 50–75% saturation with ammonium sulphate. As formaldehyde was very readily incorporated into serine and as formaldehyde also stimulated the incorporation of glycine into serine (Fig. 3), it is possible that serine biosynthesis from glycine involves a coupling of reaction (2) with reaction (1). It is possible that reaction (1) is reversible because glycine was labelled after the addition of [1-^{14}C]serine (Table 2). However, no labelling of glycine was detected when [3-^{14}C]serine was supplied to wheat-leaf extracts (Cossins & Sinha, 1965c). This suggests that reaction (2) is not readily reversible. These findings are in agreement with those reported by Wilkinson & Davies (1958), who have shown that extracts of turnip hypocotyls catalyse the interconversion of glycine and serine.

From the present studies, it appears that extracts of certain higher-plant tissues can synthesize serine from glycine without the intermediary formation of glyoxylate or formate. This biosynthesis is therefore similar in many respects to that described by Sagers & Gunsalus (1961) and by Pitts & Crosbie (1962) for certain bacteria and by Richert *et al.* (1962) for avian liver extracts.

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