Carbon Metabolism of C^{14}-Labeled Amino Acids in Wheat Leaves
I. A Pathway of Glyoxylate-Serine Metabolism [1, 2]

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Introduction

A previous report from this laboratory (22) presented evidence for the transformation of glycine-2-C^{14} into sugars in wheat leaves. However, one of the major problems that remained unanswered was the metabolic pathway by which this transformation took place. Buchanan et al. (2) showed that glycine and glycolic acid are found in the early phases of photosynthesis after the initial appearance of the sugar phosphates. Weissbach and Horecker (23) later obtained a soluble extract from spinach leaves capable of synthesizing glycine with 5-phosphoribose-1-C^{14} as the precursor. They found 80 % of the radioactivity in the α-carbon and 16 % in the carboxyl carbon of glycine. Since the spinach preparations contained pentose phosphate isomerase and transketolase they explained the incorporation of carbon-1 of ribose into the α-carbon of glycine as being due to the conversion of glycolaldehyde fragment of ribulose to glycine via glycolic and glyoxylic acids. On the other hand, Towers and Mortimer (20) studied the role of keto-acids in photosynthetic carbon dioxide assimilation and came to the conclusion that glycine and glyoxylate, respectively, arose from pyruvic acid via serine.

Tolbert and Cohen (19) obtained evidence of glycine formation from glycolate which can give rise to glyoxylate and the enzymes catalyzing the interconversion of the latter two compounds are of widespread occurrence (4, 18, 25). Accordingly it seemed reasonable to assume that the carbon atoms of glycine may be incorporated into sugars by a reversal of the reactions in the scheme of Weissbach and Horecker (23), yet none of our results could substantiate this hypothesis. This communication deals with some of the observations made during a study of the metabolic transformation of glycine to sugars in wheat leaves and a possible metabolic pathway has been proposed.

Methods & Materials

The primary leaves of green-house grown Khapli wheat seedlings were excised at the early two-leaf stage. The cut ends of excised leaves (15 leaves/treatment, having a fr wt of 0.8–0.9 g and a dry wt of 0.08–0.09 g) were allowed to stand in a vial containing feeding solution and placed in a growth chamber (21 C) under illumination (650 ft-c). During the feeding, water was added after the solution had been absorbed and subsequently whenever necessary. The feeding period varied from 10 minutes to 3 hours.

At the end of each feeding period, the leaves were immediately cut into small sections and quickly dropped into boiling 85 % ethanol; the tissues were then refluxed successively for 1 hour each, twice with 80 %, and finally with 40 % ethanol. The extracts were combined. Following the evaporation of alcohol, the extract was partitioned with chloroform to remove pigments, dried, and redissolved in 40 % ethanol. Aliquots were taken for the determination of radioactivity.

The alcoholic extract, less chloroform soluble substances, was further fractionated by means of an ion-exchange resin method (21) into neutral, acidic, and basic fractions. After the evaporation of these fractions to dryness, the residues were redissolved in 40 % ethanol and each was made up to a desired volume and aliquots were taken for the determination of radioactivity.

The neutral fraction containing essentially sugars was chromatographed in an ascending manner on Whatman No. 1 paper strips with *n*-butanol : acetic acid : water (4 : 1 : 5 v/v) as the developing solvent (12). Triple development was necessary in order to effect good separation of the sugars. The basic fraction containing essentially amino acids was chromatographed two-dimensionally in a descending manner on Whatman No. 1 paper with phenol : water (100 : 39 w/v) as the first developing solvent and *n*-butanol : acetic acid : water (100 : 22 : 50 v/v) as the second solvent (16).

Radioactive sugars were located by exposing strips of the chromatogram to Kodak No-Screen X-ray film or by scanning with an Actigraph (Nuclear Chicago). Sucrose was hydrolyzed with invertase and the products were separated chromatographically.

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[2] This work was conducted under the Extra Mural Grant No. 14 from Canada Department of Agriculture and was presented by D. Wang before the American Society of Plant Physiologists, Lafayette, Ind., in August 1961 at Purdue University.

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Keto acids were isolated as 2,4-dinitrophenylhydrazones according to the procedure of Towers and Mortimer (20). The derivatives of keto acids were separated chromatographically on Whatman No. 1 paper with *t*-amyl alcohol : ethanol : water (9 : 1 : 4 v/v) in an ascending manner in an ammonia atmosphere. The derivative of glyoxylic acid was identified by its Rf value, by cochromatography, and its characteristic color when sprayed with alcoholic sodium hydroxide solution.

► Glucose Degradation. The resulting glucose from sucrose hydrolysis was degraded by the Leuconostoc mesenteroides procedure of Gunsalus and Gibbs (6). The recovery of ethanol, lactate, and the subsequent degradation of these products were carried out according to the experimental conditions given by Canvin and Beevers (3). The ethanol was recovered by distillation and oxidized to acetic acid (1). Lactic acid was extracted from the residue with ether following acidification and then degraded to CO_2 and acetic acid (9). Acetic acid was recovered by steam distillation, converted to its silver salt by the method of Jourdian (7), and degraded by Schmidt reaction in a Stutz and Burris apparatus (17) in which the zinc trap was replaced with a permanganate scrubber according to the experimental conditions given by Canvin and Beevers (3). The water soluble compounds were oxidized to CO_2 with persulfate (8).

► Assay of Radioactivity. All radioactive fractions except $BaC^{14}O_3$ were assayed by counting samples with infinite thinness with a Nuclear Chicago micromil window gas flow G-M counter. No correction for back scattering was made. Carbon dioxide derived from glucose degradation was assayed as $BaCO_3$ on sintered porcelain disks with the use of a Mylar window gas flow G-M counter. The counts were corrected for background and self absorption, the latter correction was made for $BaC^{14}O_3$ assay only.

The carbon-14-labeled compounds used in this study are glycine-1-C^{14} (0.1 mc/6.5 mg), glycine-2-C^{14} (0.1 mc/1.53 mg), serine-3-C^{14} (0.1 mc/1.2 mg) obtained from Merck and Co., Montreal and glyoxylic acid-1,2-C^{14} (3 mc/mmole) from California Corp. for Biochemical Research, Los Angeles. The level of radioactivity of labeled compounds used in the experiments reported here was 2 μc per treatment, and the concentration of non-radioactive compound used in isotopic competition experiments was ten times that of the labeled substrate.

Experimental Results

To test the hypothesis that the carbon metabolism of glycine follows a reversal of the reaction scheme of Weissbach and Horecker (23), the isotopic competition technique was used. If the hypothesis is correct, the suspected intermediates, glycolate and glyoxylate, should markedly lower the conversion of the substrate to sugars when fed together with labeled glycine. Table I shows the effect of non-radioactive glycolate and glyoxylate on the formation of sugars from glycine-2-C^{14}. Apparently, neither compound decreased the amount of isotope going into sugars. On the contrary, both enhanced the rate of flow of the isotope. The results also held true when feeding times were varied; the percentage ratio of glycine-2-C^{14} plus glycolate to glycine-2-C^{14} was 1.5 and 2.6 at the end of 60 and 180 minutes, respectively, and that of glycine-2-C^{14} plus glyoxylate to glycine-2-C^{14} was 1.5 and 1.6 at the end of 10 and 30 minutes. A similar effect on the conversion of glycine-1-C^{14} to sugars is evident from table I. It is also clear that wheat leaves could utilize both carbon atoms of this amino acid equally well for the synthesis of sugars in light. The increased radioactivity of sugars was accompanied by a concomitant decrease of the isotope in the amino acids.

The synthesis of sugars from carbon-14-labeled glycine was much less in darkness than in light. Despite the lower incorporation of label into sugars the effect of glycolate and glyoxylate was still evident although their major effect was on the transfer of label to the organic acid fraction rather than to sugars.

The uncoupling agent 2,4-dinitrophenol, which has been shown to decrease the photosynthetic assimilation of $C^{14}O_2$ by tobacco leaves (11), exhibited only a slight effect on the carbon metabolism of gly-

Table I

Effect of Glycolate & Glyoxylate on Carbon Metabolism of Glycine-C^{14} in Detached Leaves of Khapli Wheat in Light*

Fraction	Radioactivity in cpm/g fresh tissue									
	Glycine-1-C^{14}		Glycine-1-C^{14} + glycolate		Glycine-2-C^{14}		Glycine-2-C^{14} + glycolate		Glycine-2-C^{14} + glyoxylate	
		%		%		%		%		%
Alcohol soluble (less $HCCl_3$ soluble)	66,200	100.0	93,000	100.0	124,200	100.0	54,700	100.0	111,000	100.0
Amino acids**	46,500	70.0	38,800	41.7	76,400	61.5	29,500	54.0	64,000	58.0
Organic acids**	4,100	6.2	17,800	19.1	9,600	7.7	2,600	4.8	12,300	11.8
Sugars**	13,200	19.5	36,400	39.5	31,800	25.7	22,500	41.1	41,300	37.5

* Feeding period was 60 minutes.

** These fractions correspond to the total radioactivities of the basic, acidic, and neutral fractions derived from the treatments with ion-exchange resin.

Table II

Effect of Glycolate & Glyoxylate on Carbon Metabolism of C^{14}-Labeled Glycine in Detached Leaves of Khapli Wheat in Darkness*

Fraction	Radioactivity in cpm/g fresh tissue											
	Glycine-1-C^{14}		Glycine-1-C^{14} + glycolate		Glycine-1-C^{14} + glyoxylate		Glycine-2-C^{14}		Glycine-2-C^{14} + glycolate		Glycine-2-C^{14} + glyoxylate	
		%		%		%		%		%		%
Amino acids	48,000 36,800	94.1 89.7	21,600	56.7	21,100	77.0	86,000 54,500	95.4 89.3	37,300	53.2	41,000	51.4
Organic acids	2,200 3,200	4.3 8.5	14,500	38.1	5,100	18.8	3,100 4,500	3.4 7.4	30,500	43.4	35,300	44.2
Sugars	800 700	1.6 1.9	2,000	5.2	1,000	3.7	1,000 2,000	1.1 3.3	2,400	3.4	3,500	4.4

* Feeding period was 120 minutes. The percentage in this table was calculated from the sum total of these three fractions that was taken as 100.

Table III

Effect of Dinitrophenol on Carbon Metabolism of Glycine-2-C^{14} in Detached Leaves of Khapli Wheat in Light*

Fraction	Radioactivity in cpm/g fresh tissue							
	Glycine-2-C^{14}		Glycine-2-C^{14} + DNP**		Glycine-2-C^{14} + glyoxylate		Glycine-2-C^{14} + glyoxylate + DNP**	
		%		%		%		%
Alcohol soluble (less $HCCl_3$ soluble)	143,500	100.0	183,800	100.0	174,000	100.0	177,000	100.0
Amino acids	59,430	41.3	64,000	34.8	44,830	25.8	82,250	46.4
Organic acids	9,570	6.7	12,910	7.0	12,650	7.3	12,640	7.2
Sugars	70,950	48.4	81,050	44.1	97,900	56.3	69,130	39.1

* Feeding period was 180 minutes.
** The concentration of DNP was 10^{-3} M.

cine (table III). It did, however, overcome the effect of glyoxylate on the conversion of glycine to sugars.

In a preliminary experiment using glyoxylate-1,2-C^{14} as the substrate, it was found that it could serve as an effective precursor for the synthesis of sugars. Furthermore, paper chromatographic examination of the amino acids which resulted from feeding wheat leaves with either glyoxylate-1,2-C^{14}, or glycine-1-C^{14}, or glycine-2-C^{14} indicated a similar pattern of radioactivity, the most heavily labeled compounds being serine and glycine. Other experiments indicated that the incorporation of the label from glycine-2-C^{14} into the keto acids of either leaves or leaf homogenates was not affected by glyoxylate (table IV). Although one of the duplicates in the leaf homogenate experiment had a higher value, radioautographs of the keto-acid derivatives did not reveal any difference in the relative radioactivity of glyoxylate between treatments.

The above observations suggest that the conversion of glycine to glyoxylate may not constitute the main pathway in glycine catabolism. This led us to investigate the possible existence of an alternative

Table IV

Effect of Glyoxylate on Formation of Keto-Acids* From Glycine-2-C^{14}

Tissue	Incubation period	Radioactivity	
		Glycine-2-C^{14} cpm**	Glycine-2-C^{14} + glyoxylate cpm**
Detached primary leaves***	3 hrs	320	310
Leaf homogenate†	45 min	340	280 290 620

* Isolated from either the leaves or the leaf homogenate as 2,4-dinitrophenylhydrazone derivatives.
** Figures represent counts per minute per treatment.
*** 20 leaves (1.4 g fr wt)/treatment were used.
† The system consists of 5 ml of leaf homogenate (from 20 g leaves ground in 40 ml of 0.2 M phosphate buffer, pH 7,), 2 μc of glycine-2-C^{14}, and glyoxylate (10 times the concentration of the labeled glycine) when required. The reaction mixture was incubated at 30 C and reaction was terminated by adding 2,4-dinitrophenylhydrazine reagent.

route in the oxidation of glycine and the possibility of the conversion of glyoxylate to sugars by way of glycine.

Further experiments with glyoxylate-1,2-C^{14} revealed that in the presence of non-radioactive glycine or serine, the amount of isotope incorporated into sugars was decreased to about half that of the control at the end of 30 minutes (table V) and the decreased incorporation of radioactivity was still evident after 3 hours. Moreover, the rate of incorporation of the isotope from glycine-2-C^{14} was also curtailed by simultaneous feeding with non-radioactive serine or formate (table VI). On the other hand, 3-phosphoglyceric acid showed some degree of enhancement. These results strongly suggest that glycine and serine lie on the main pathway of the synthesis of sugars from glyoxylic acid.

When serine-3-C^{14} was the substrate, the presence of non-radioactive glycine did not affect the rate of sugar synthesis, in fact, it exhibited a slight stimulatory effect (table VII). However, in the presence of glyoxylic acid the synthesis of sugars from serine-3-C^{14} was almost doubled (table VII).

The glucose moiety of sucrose was degraded to determine the intramolecular distribution of the isotope. When glycine-2-C^{14} was the substrate, the carbon 14 was mainly distributed among carbons 1, 2, 5, and 6 (table VIII). With glycine-1-C^{14} as the substrate 76.2 % of the isotope was located in carbons 1, 3, 4, and 6 and 23.8 % in carbons 2 and 5 (table VIII).

Table V

Effect of Glycine & Serine on Formation of Sugars From Glyoxylate-1,2-C^{14} in Detached Leaves of Khapli Wheat in Light

Experiment No.	Feeding period min	Radioactivity in cpm/g fresh tissue					
		Glyoxylate-1,2-C^{14}		Glyoxylate-1,2-C^{14} + glycine		Glyoxylate-1,2-C^{14} + serine	
			%*		%*		%*
1	30	32,300	27.3	16,500	8.2	24,900	13.5
		47,800	21.3	20,000	12.5		
2	180	72,140	55.0	56,640	48.5		
		64,140	53.6	54,240	45.8		
				43,940	46.0		

* The radioactivity in the corresponding alcohol soluble fraction constitutes 100 %.

Table VI

Effect of Serine, Formate, & Phosphoglycerate on Formation of Sugars From Glycine-2-C^{14} in Detached Leaves of Khapli Wheat in Light

Experiment No.	Feeding period min	Radioactivity in cpm/g fresh tissue							
		Glycine-2-C^{14}		Glycine-2-C^{14} + serine		Glycine-2-C^{14} + formate		Glycine-2-C^{14} + 3-phosphoglycerate	
			%*		%*		%*		%*
1	30	21,380	10.6	8,320	4.9				
2	30	40,490	13.7	24,090	9.9	30,290	10.8	30,290	18.5
						24,990	9.1		

* See footnote of table V.

Table VII

Effect of Glyoxylate & Glycine on Formation of Sugars From Serine-3-C^{14} in Detached Leaves of Khapli Wheat in Light

Experiment No.	Feeding period min	Radioactivity in cpm/g fresh tissue					
		Serine-3-C^{14}		Serine-3-C^{14} + glyoxylate		Serine-3-C^{14} + glycine	
			%*		%*		%*
1	30	9,640	4.8	24,140	10.3	11,840	5.7
2	30	18,190	5.5	24,490	10.0		
3	30	3,550	3.7			4,900	5.1
						3,600	4.2

* See footnote of table V

Table VIII

% Distribution (Based on Specific Activity) of C^{14} in Glucose Moiety of Sucrose Isolated From Wheat Leaves Fed With Glycine-2-C^{14} & Glycine-1-C^{14}

Carbon No. of Glucose	Glycine-2-C^{14}					Glycine-1-C^{41}
	1	2	3	4*	5**	1
1	30.3	32.4	30.2	29.9	28.0***	20.7
2	23.1	27.9	23.6	19.6	26.3	15.6
3	2.2	4.1	3.3	4.2	5.7	19.4
4	2.8	2.5	2.7	7.2	7.1	21.4
5	24.8	15.6	20.2	24.8	16.2	8.2
6	16.7	17.7	19.8	14.3	16.7	14.7

* Feeding solution contained non-radioactive erythrose.
** Feeding solution contained 10^{-3} M DNP.
*** Cross contamination error within 5 %.

With glyoxylic-1,2-C^{14} acid as the substrate, the major radioactivity was found in carbons 1, 2, 5, and 6, and carbons 3 and 4 contained about 17 % of the total (table IX). These results indicate glucose was formed by a condensation of two 3-carbon compounds.

Some of the enzymes involved in the metabolism of these 2-carbon compounds in wheat leaves of the Khapli variety have also been under investigation. Glycolic acid oxidase, which catalyzes the conversion of glycolate to glyoxylate, is present, but in comparison to the enzyme from leaves of tobacco or spinach it is considerably less saturated with respect to its coenzyme riboflavin phosphate. Partially purified preparations freed from catalase evolve carbon dioxide. In addition, a DPNH linked glyoxylate reductase and glyceric dehydrogenase are present similar to that found in other plant tissues and wheat (15, 25), respectively. The enzyme catalyzing the glycine-serine conversion awaits demonstration, but a transaminase that catalyzes the transfer of the amino group of serine to glyoxylate has been demonstrated in crude dialyzed extracts of leaves. The products of the enzymic reaction are glycine and an α-keto acid, the 2,4-dinitrophenylhydrazone of which upon catalytic hydrogenation yields a ninhydrin positive compound having the chromatographic properties of serine. Presumably the α-keto acid is β-hydroxypyruvate, although the reverse reaction has not been demonstrated as yet. The properties of the enzyme are now under investigation.

Table IX

% Distribution (Based on Specific Activity) of C^{14} in Glucose Moiety of Sucrose Isolated From Wheat Leaves Fed With Glyoxylic-1,2-C^{14} Acid

Carbon No. of glucose	Glyoxylic-1,2-C^{14} acid	
	1	2
1	28.6	30.7
2	17.8	20.2
3	8.3	9.0
4	9.2	7.7
5	16.9	14.2
6	19.1	18.6

Discussion

In higher plants glycine can readily arise from either carbon dioxide (2, 11), ribose-5-phosphate (23), glycolate (19), or glyoxylic-1,2-C^{14} acid. Because of the wide occurrence of transaminases (10) it is generally accepted that the mechanism of the oxidation of glycine is generally by its conversion to glyoxylic acid. Since wheat leaves readily utilize the α-carbon atom of glycine for the synthesis of sugars (22), the possibility could not be excluded that the carbon atoms of this amino acid may be incorporated into sugars by means of the reversal of the reaction scheme of Weissbach and Horecker (23). However, this was rendered unlikely by the observations that neither non-radioactive glycolic nor glyoxylic acid decreased the amount of isotope that was incorporated into sugars from glycine. This indicates that neither of the former compounds lies on the main pathway from glycine to sugars, unless exogenous glyoxylic or glycolic acid does not mix with endogenous acid formed from glycine. Such a situation might exist because of compartment barriers within the cell or the utilization of the metabolites in an activated form. However, the enhancement of the movement of label from glycine to sugars by either glycolic or glyoxylic acid can hardly be reconciled with the foregoing argument, and the lack of appreciable conversion of glycine to glyoxylic acid reflected by the radioactivity in the latter would strengthen the argument that neither glycolic nor glyoxylic acid lies on the main pathway in the oxidation of glycine. On the other hand, the conversion of glyoxylic-1,2-C^{14} acid to sugars was drastically curtailed by the presence of non-radioactive glycine or serine. Non-radioactive serine also lowered the synthesis of sugars from glycine-2-C^{14} but the reverse was not true. However, the possible participation of a limited conversion of glycine to glyoxylic acid, which will be considered later, cannot be excluded.

These results strongly suggest that glyoxylic acid is converted to glycine and then to serine in the synthesis of sugars. Since glycolic acid is readily oxidized to glyoxylic acid (18) the stimulatory effect of glyoxylate may be due to the following.

► I. Glyoxylate gives rise to formate and CO_2 (4), the former then being activated and utilized in the formation of serine from glycine (14, 24). The relatively small decrease in sugar synthesis from glycine-2-C^{14} in the presence of non-radioactive formate indicates a limited activation of exogenous formate in wheat leaves.

► II. Glyoxylate also serves as an acceptor for the amino-group of serine producing glycine and β-hydroxypyruvic acid according to the scheme in figure 1. The demonstration of a serine-glyoxylate transaminase in wheat leaves provides enzymatic evidence in support of this hypothesis.

Hydroxypyruvic acid may be reduced to glyceric acid which is subsequently used in glucose synthesis. The enzyme, glyceric dehydrogenase, catalyzing this

reaction has been demonstrated in wheat leaves by Stafford et al. (15) and it was also found to be highly active in the wheat leaves that we used in these experiments. The equilibrium of this reaction was also reported to be in favor of the formation of glyceric acid. Dickens (5) has also reported the conversion of 3-C-14- and 2-C-14-hydroxypyruvate to the hexose of glycogen in rat livers. The pattern of labelling in his experiments indicated the condensation of two 3-carbon compounds, presumably glyceric acid, arising from hydroxypyruvate. However this was not a major pathway for the metabolism of hydroxypyruvate in rat liver.

The intramolecular distribution of isotope in the glucose moiety of sucrose which resulted from feeding leaves with glycine-1-C^{14} and -2-C^{14} (table VIII) is consistent with the idea that glucose is synthesized by the condensation of two 3-carbon compounds derived from serine. Carbons 3 and 4 of glucose contained the lowest percentage of isotope with glycine-2-C^{14} whereas with glycine-1-C^{14} carbons 3 and 4 retained more isotope than either carbons 2 and 5 or carbons 1 and 6. There appears to be a high degree of randomization between carbons 2 and 5 and carbons 3 and 4 in glucose when glycine-1-C^{14} is fed in contrast to the lower degree of randomization with glycine-2-C^{14}. This unequal degree of randomization owing to the different origins of these carbon atoms is also seen in the distribution of isotope between carbons 3 and 4 and carbons 2 and 5 in glucose when leaves were fed with uniformly labeled glyoxylate (table IX). No explanation is offered for the almost twofold higher labelling of carbon 1 as compared to carbon 6. These studies were carried out in light and in relatively long term experiments which would permit a certain amount of recycling of the intermediates and end products through other metabolic pathways.

Nevertheless, the results from the isotopic competition experiments indicate the pathway of synthesis of sugars as follows: glyoxylate——>glycine——>serine——>hexose. According to this scheme hexose is derived by a condensation of two 3-carbon compounds derived from serine and the distribution of the carbon atoms of serine in glucose would be as follows:

$$\begin{array}{llllllllllll} & & & & & & & 1 & 2 & 3 & 4 & 5 & 6 \\ \beta & \alpha & C & & C & \alpha & \beta & \beta & \alpha & C & C & \alpha & \beta \end{array}$$

$$C-C-C + C-C-C \longrightarrow C-C-C-C-C-C$$

where α, β, and C(COOH) are the respective carbon atoms of serine.

The relatively high percentage distribution of isotope in carbons 1 and 6 of glucose after feeding carbon-14 labeled glycine and glyoxylate-1-2-C^{14} indicates that a fraction of the exogenous glycine and glyoxylate has access to the formyl activating sites and that both the α and carboxyl carbons of glycine give rise to the β carbon of serine. The conversion of the α-carbon of glycine to carbons 1 and 6 of glucose may be accounted for either by a direct cleavage of glycine to a one carbon unit and CO_2 (13) or by transamination of glycine to glyoxylate which is then oxidized to CO_2 and a formyl group. The pathway which converts the carboxyl carbon of glycine to the β carbon of serine is more difficult to visualize. The possibility of recycling following its oxidation to CO_2 through photosynthesis which could hardly account for the extent of the labelling at this light intensity cannot be entirely excluded. There is also the possibility of glycine recycling through purines to allantoic acid and subsequently glyoxylate in which the α and carboxyl carbons of glycine are exchanged.

From this study, we are led to conclude that glycine and serine under the experimental conditions described here, are synthesized through one metabolic pathway and catabolized via another. On the basis of these findings and other published results a metabolic pathway is proposed as shown in figure 1. This pathway may indeed play a major role in the metabolism of glycine and serine in wheat.

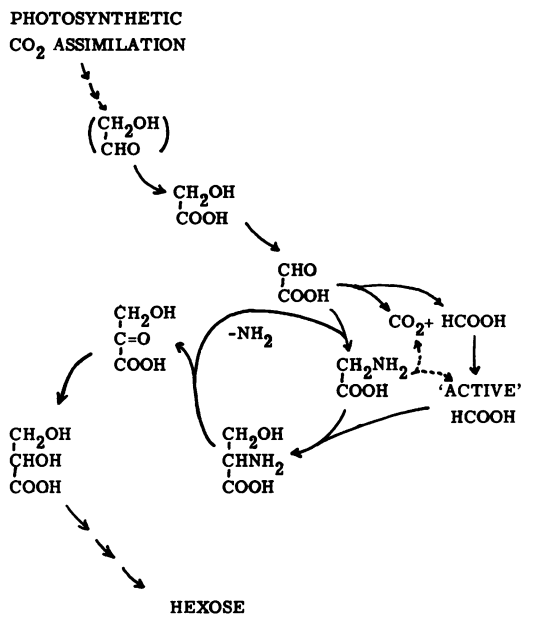

Fig. 1. A pathway of glyoxylate-serine metabolism.

Summary

I. In isotopic competition experiments neither glycolate nor glyoxylate could lower the radioactivity in sugars when glycine-C^{14} or serine-3-C^{14} was used as the substrate. On the contrary, both compounds greatly enhanced the rate of transformation from glycine-C^{14} to sugars.

II. The formation of sugars from glyoxylate-C^{14} was greatly reduced by the addition of either glycine or serine.

III. The synthesis of sugars from glycine was reduced by serine but not vice versa.

IV. No difference was found in radioactivity between 2,4-dinitrophenylhydrazones of glyoxylate derived from feeding with glycine-2-C^{14} alone and from glycine-2-C^{14} plus glyoxylate.

V. The intramolecular distribution of C^{14} in the glucose moiety of sucrose derived from labeled glycine-1-C^{14}, glycine-2-C^{14}, or glyoxylate-C^{14} suggests that glucose was formed by the condensation of two 3-carbon compounds.

VI. On the basis of these findings a pathway of glyoxylate-serine metabolism in wheat leaves is proposed.

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