A STUDY OF RIBOSE METABOLISM IN THE TOBACCO PLANT

By

Thomas Griffith, Jr.

A THESIS

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT

These investigations were conducted to study the synthesis of methyl groups from photosynthetic processes in higher plants. D-Ribose-1-C^{14} was administered to tobacco plants and to detached tobacco plant leaves in order to study the incorporation of C^{14} into nicotine, glycine and serine.

D-Ribose-1-C^{14} was incorporated into the nicotine molecule although the methyl group of nicotine contained only a small part of the C^{14}. When tobacco plants were allowed to metabolize the radioactive pentose for two, three or seven days, the C^{14} concentration of the nicotine was similar. However, the C^{14} located in the methyl groups decreased with the shorter metabolism period. The rate and great variety of the metabolic transformations of pentoses and pentose derivatives in plants precludes the possibility that a large quantity of the D-ribose-1-C^{14} would be utilized for synthesis of one carbon units. The rapid synthesis of nicotine-C^{14} suggested that the pyridine ring of nicotine was formed from a compound related to glycolysis.

D-Ribose-1-C^{14} was administered to detached tobacco plant leaves. Serine-C^{14} was synthesized during dark metabolism but no radioactive glycine was detected. The C^{14} was located primarily in the beta carbon of the serine with a lesser quantity in the alpha carbon. Serine synthesized during light metabolism contained the majority of the C^{14} in the alpha and beta carbons. The specific activity of the beta carbon

was greater than that of the alpha carbon. Glycine synthesized during light metabolism was labeled predominantly in the alpha carbon. A mechanism for glycine and serine synthesis which involved two metabolic pathways was postulated. It was suggested that glycine and serine were synthesized mainly from glyoxylic acid which was formed in the glyoxylate cycle and to a lesser extent by conversion of 3-phosphoglyceric acid to serine.

The isolation of glycine and serine containing C^{14} in the alpha and beta carbons showed one possible pathway by which methyl groups can be synthesized from the photosynthetic cycle.

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INTRODUCTION

INTRODUCTION

The transfer of methyl groups was established as a reaction in living organisms by du Vigneaud *et al.* (1) in 1940. Since this time much attention has been focused on the transfer and biosynthesis of the one carbon unit. Organic acids, amino acids, and other types of compounds have been shown to be precursors of methyl groups in plants and animals (1-16).

The only carbon source necessary for growth in most higher plants is carbon dioxide. However, no evidence has been reported that carbon dioxide can be reduced directly to a methyl group. Also, no compound formed during the photosynthetic cycle has been shown to be a methyl group precursor. A pathway by which methyl groups may be formed from carbon dioxide in plants is yet to be demonstrated.

Previous work in this laboratory has shown that the nicotine methyl group may be synthesized by transmethylation reactions and by reduction of a one-carbon intermediate believed to be an active or bound form of formaldehyde. The "active one-carbon unit" was synthesized from the alpha carbon of glycine, the alpha carbon of glycolic acid, and the beta carbon of serine. These compounds are labeled rapidly during the photosynthesis of $C^{14}O_2$ and are believed to arise from a common precursor formed in the photosynthetic cycle.

This study was undertaken to demonstrate a pathway for the formation of glycine and serine from the photosynthetic cycle.

The formation of methyl groups from photosynthetic carbon dioxide could be explained with this information. D-Ribose-C^{14} was administered to tobacco plants and detached leaves of tobacco plants. Nicotine, glycine and serine were isolated and degraded. The distribution of C^{14} in these molecules was determined.

HISTORICAL

HISTORICAL

The transfer of the methionine methyl carbon to an oxygen or nitrogen atom of another metabolite has been reported from several laboratories (1-8). The methionine methyl group, labeled with C^{14}, was incorporated into nicotine in tobacco plants (2). All the C^{14} was located in the methyl group of the nicotine. Dewey et al. (3), using methionine containing C^{14} and deuterium in the methyl group, showed the transmethylation reaction occurred with no intermediate oxidation and reduction during the transfer. Sato et al. (4) supported these results by showing that the methyl group of methionine was transferred intact to form the methyl esters of pectinic acid in radish plants. Also, methionine was shown to be a methyl donor in the formation of lignin of tobacco plants (5), ricinine of castor beans (6), and hordenine of barley (7). Glycine betaine and choline are metabolites in plants which contain three N-methyl groups. The methyl groups of glycine betaine were introduced into nicotine to an extent approximately equal to that of the methionine methyl group (9). Glycine betaine has been shown to be a methyl donor for the formation of N-methyltyramine and hordenine of barley plants (10). The methyl groups of choline were not incorporated into the barley plant alkaloids or ricinine from the castor bean although the methionine methyl carbon was utilized to form choline methyl groups (6,11). Byerrum et al. (9) has shown that choline methyl groups were precursors of the N-methyl

group in nicotine and incorporated to an extent similar to that of the methyl carbons of methionine and glycine betaine.

Several metabolites labeled with C^{14} have been administered to plants in an attempt to establish the origin of methyl groups and the mechanism of their transfer and reduction. The alpha carbon of glycine (12), the alpha carbon of glycolic acid (13), and the beta carbon of serine (14) were shown to be utilized by tobacco plants for the formation of the nicotine methyl group. They were incorporated to an extent similar to that of the methyl groups of methionine, glycine betaine and choline. These carbon atoms were shown also to be incorporated into the methoxyl groups of lignin from tobacco plants (15) and methyl esters of pectinic acid from radish plants (16). It is significant that glycine, glycolic acid and serine contribute to the one-carbon pool and, also, are among the first products to be labeled when plants photosynthesize $C^{14}O_2$ (17-22).

The hypothesis that the one-carbon unit of glycine, serine and glycolic acid was transferred at the oxidation state of formic acid or formaldehyde has been tested. Formic acid was shown to be metabolized and reduced to the methyl group of nicotine from tobacco plants (2), pectinic acid from radish plants (16), hordenine from barley plants (7), ricinine from castor beans (6), and lignin from tobacco plants (5). The incorporation into nicotine was only one-tenth the extent as that of the methionine methyl carbon. Formic acid did not appear to be an intermediate in the transfer of one carbon units in tobacco plants because it was a very poor precursor of methyl groups. Byerrum *et al.*

(14) fed tobacco plants formaldehyde-C^{14}. The methyl group of nicotine contained three to four times more C^{14} than the nicotine from corresponding experiments with glycine-2-C^{14}, serine-3-C^{14}, or glycolic acid-2-C^{14}. These data supported the hypothesis that the one carbon unit is transferred at the formaldehyde oxidation state. The role of formaldehyde in methyl group synthesis appears to be limited to the transport mechanism rather than as a primary source of methyl carbons since free formaldehyde is not a normal constituent in plants.

A pathway for formation of one-carbon units or methyl groups from photosynthetic carbon dioxide is not known. No evidence has been reported that carbon dioxide can be reduced directly to contribute to the one-carbon unit pool. Culp (23) and Ganz et al. (24) administered $C^{14}O_2$ and $NaHC^{14}O_3$ to tobacco plants and isolated nicotine containing C^{14}. Culp found that the N-methyl group contained only 13% of the radioactive carbon in the molecule. If random labeling occurred, one would expect 10% of the C^{14} in the methyl group as there are ten carbon atoms in nicotine. Dubeck and Kirkwood (6) found no C^{14} in the O- or N-methyl groups of ricinine when barley plants were allowed to metabolize $C^{14}O_2$. Kuzin and Merenova (25) administered radioactive carbon dioxide to excised tobacco plant leaves and found all the C^{14} to be in the methyl group. They believed that carbon dioxide could be incorporated specifically into the methyl group. However, Dawson (26) and Tso and Jeffrey (27) have shown that the main site of nicotine synthesis is in the root although the transfer of methyl groups to the nicotine ring

system may occur in the leaves. Thus, the only probable location of C^{14} would have been the methyl group.

The photosynthetic fixation of carbon dioxide by plants has been studied extensively in recent years. The first stable product in which C^{14} appears during photosynthesis with $C^{14}O_2$ is 3-phosphoglyceric acid (28). The photosynthetic fixation reaction has been shown to be a chemical combination of ribulose diphosphate and carbon dioxide to form two molecules of 3-phosphoglyceric acid (29-32). Ichihara and Greenberg (33) obtained enzymes from rat liver which formed serine from 3-phosphoglyceric acid via phosphohydroxypyruvic acid and phosphoserine. Also, Vernon and Arnoff (17) and Newburgh and Burris (34) proposed that a similar type of reaction occurred in plants. They postulated the conversion of alanine to serine without rupture of any carbon-carbon bonds. This scheme would constitute a fairly direct conversion of respiratory carbon dioxide into methyl groups. Frontera-Aymat (35) fed tobacco plants pyruvic acid-3-C^{14}. She isolated radioactive nicotine but only 6% of the C^{14} in the molecule was located in the methyl group. In contrast, the beta carbon of serine was incorporated specifically into the methyl group of nicotine (14). Tolbert and Gailey (36) studied $C^{14}O_2$ fixation by etiolated wheat plants. Alanine and 3-phosphoglyceric acid accumulated large amounts of C^{14} during the initial period of photosynthesis. Serine and glycine were not labeled substantially during the first 20 hours of photosynthesis. The incorporation of C^{14} into glycine and serine coincided with the

formation of choloroplasts and activation of glycolic acid oxidase. These data indicated that serine did not arise from a triose or other three carbon compound from the photosynthetic cycle.

Glycolic acid, serine and glycine appear to be interrelated in plant metabolism (18,37). Other studies have demonstrated the interconversion of serine and glycine (38,39). Tolbert and Cohen (18) have shown that barley and wheat leaves convert the two carbon atoms of glycolic acid directly into glycine and the carboxyl and alpha carbons of serine. The beta carbon of serine was derived in part from the alpha carbon of glycolic acid. The accumulation of C^{14} in glycolic acid (20-22) and glycine and serine (17-20) during short time photosynthesis has been observed.

The interrelationship of glycine, serine and glycolic acid has been established. However, the formation of these metabolites from the photosynthetic cycle is uncertain. Calvin and co-workers (21,28) have observed a marked increase in glycolic acid formation during photosynthesis in the absence of carbon dioxide. Also, the carbon atoms of glycolic acid and the alpha and beta carbons of 3-phosphoglyceric acid were uniformly labeled when plants photosynthesized carbon dioxide-C^{14} (37,40). It is believed that glycolic acid is related to the photosynthetic cycle and glycine and serine are formed from glycolic acid.

Smith and Gunsalus (41) discovered an enzyme, isocitrase, that cleaved isocitric acid to glyoxylic acid and succinic acid. The enzyme was purified from extracts of P. aeruginosa. Kornberg and Krebs (42)

extended these studies and proposed a new metabolic cycle to occur in conjunction with the citric acid cycle. The glyoxylate cycle consists of the cleavage of isocitric acid to glyoxylic acid and succinic acid and the condensation of acetate and glyoxylic acid to malic acid. The latter reaction is catalyzed by the enzyme malate synthetase (43). Recently Beavers and Kornberg (44,45) demonstrated the presence of these enzymes in castor bean plants. The importance of this pathway for glycolic acid formation is not known. The distribution of C^{14} in glycolate during photosynthesis with $C^{14}O_2$ does not negate this mechanism.

Wilson and Calvin (29) have attributed glycolic acid formation in plants to a cleavage of the glycolyl-transketolase complex. This enzyme catalyzes the transfer of a glycolyl group to an appropriate aldehyde acceptor to form a ketose (46-48). The formation of pentose phosphates in plants are believed to occur primarily by this pathway (28,49). The formation of xylulose-5-phosphate from fructose-6-phosphate and glyceraldehyde-3-phosphate and the formation of ribose-5-phosphate and xylulose-5-phosphate from sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate involves a transketolase enzyme which transfers a glycolyl fragment. During photosynthesis the utilization of pentose phosphates is rapid. The forward reaction is predominant due to the constant depletion of pentose phosphates which are necessary for fixing carbon dioxide. If the reverse reaction was forced, the glycolyl-enzyme complex and glyceraldehyde-3-phosphate would compete for erythrose-4-phosphate. The erythrose-4-phosphate pool is very small

(not chromatographically detectable) and competition for the small quantity of tetrose phosphate will increase the mean lifetime of the glycolyl-enzyme complex. Wilson and Calvin (29) illuminated plants in the absence of carbon dioxide and observed a rapid accumulation of pentose phosphates. After a short period glycolic acid concentration increased and the pentose phosphate concentration decreased. The increase in glycolic acid was believed to be due to the increase in glycolyl-enzyme concentration and its subsequent decomposition to glycolic acid and free enzyme. This mechanism is consistent with the available information concerning glycolic acid formation.

Greenberg and Sassenrath (50) investigated the significance of this metabolic pathway in rats. They fed rats ribose-1-C^{14} and arabinose-1-C^{14}. Benzoic acid was ingested along with the pentoses and glycine was isolated from urine as hippuric acid. No C^{14} was detected in the hippuric acid in either experiment. Weissbach and Horecker (19), using a soluble extract from spinach leaves, reported the conversion of 1-C^{14}-ribose-5-phosphate to glycine. The glycine was labeled predominantly in the alpha carbon.

EXPERIMENTAL AND RESULTS

EXPERIMENTAL AND RESULTS

Preparation of Plants

The tobacco plants used in this study were Nicotiana rustica L. var. humilus, a strain with a high nicotine content. The seeds were planted in flats containing vermiculite, a commercial non-nutrient, supporting material. After two weeks the seedlings were transplanted to insure sufficient separation in the flats. The plants were fed twice a week with a nutrient solution composed of 5.8 g. $Ca(NO_3)_2 \cdot 4H_2O$, 1 g. $MgSO_4 \cdot 7H_2O$ and 1 g. K_2HPO_4 in 4 liters of water. Additional water was administered as required. The plants were grown in the green house until a height of 6 inches was attained. The growing period was about 90 days.

To prepare plants for hydroponic administration of the radioactive compound, they were removed from the flats and the roots rinsed carefully with tap water to remove the adhering vermiculite. The roots were soaked in a 0.1 percent solution of a detergent germicide (Detergent germicide No. 1528 manufactured by Wyandotte Chemical Co., Wyandotte, Michigan) for an hour with occasional agitation. The roots were rinsed free of germicide with tap water and placed in 125 ml. Erlenmeyer flasks containing 50 ml. of an inorganic nutrient solution. The nutrient solution was a 1:3 dilution of the stock solution whose composition is shown in Table I. The nutrient solution was saturated

TABLE I

COMPOSITION OF THE STOCK NUTRIENT SOLUTION

Water	1000 ml.	Magnesium sulfate	250 mg.
Calcium nitrate	1 g.	Ammonium sulfate	250 mg.
Potassium chloride	250 mg.	Potassium dihydrogen phosphate	250 mg.
Ferric chloride	2 mg.		

with oxygen gas to provide aerobic conditions for the roots. Three drops of 1 percent solution of detergent germicide was added to each flask to protect against possible microbial destruction of the radioactive compound during the feeding period.

The plants were grown in a laboratory fume hood following the administration of D-ribose-1-C^{14}. Artificial lights were used during the administration period. Two 36 inch 30 watt fluorescent tubes and one 100 watt incandescent bulb was placed 14 inches above the plants. The light intensity at the top of the plants was about 200 footcandles. The plants were illuminated 12 hours per day.

Isolation and Purification of Nicotine

Nicotine was isolated from tobacco plants following the administration of D-ribose-1-C^{14}. The plants were removed from the nutrient solution and the roots rinsed with tap water. The plants were cut into small pieces and dried under a heat lamp at 80° C. for 6 hours. The dry plant material was ground with a small mortar and pestle and transferred to a micro-Kjeldahl flask containing four drops of 1 percent G. E. antifoam solution (G. E. antifoam 60, General Electric Co.,

Schnectedy, N. Y.). The nicotine was separated by steam distillation and the distillate collected in 1 ml. of 6 M HCl. The steam distillation was continued until no additional nicotine was in the distillate. The distillate was tested with silicotungstic acid which forms a white, insoluble salt with nicotine. The distillate was concentrated *in vacuo* to a small volume and the nicotine purified by two successive azeotropic distillations from a basic solution into 1 ml. of 6 M HCl using a Widmar column (51). After purification, the water and excess hydrochloric acid was removed *in vacuo* and residual nicotine hydrochloride dissolved in a small quantity of water and methanol. A saturated solution of picric acid in methanol was added in excess and the nicotine dipicrate allowed to crystallize. The precipitate was collected and recrystallized from water. The melting point was 225-227° C. A reported value is 224° C. (52).

Demethylation of Nicotine

Nicotine isolated from plants fed D-ribose-1-C^{14} was radioactive. The methyl group of nicotine was isolated as methyltriethylammonium iodide by the procedure of Pregl (53) as modified by Brown and Byerrum (2). The quatenary ammonium salt was counted to determine the quantity of C^{14} in the methyl group.

An additional modification of the demethylation procedure was used. Brown and Byerrum chose to demethylate nicotine hydrochloride because the dipicrate derivative is extremely insoluble in acid solvents. In this study only small quantities of nicotine were isolated from

plants and it was desirable to eliminate unnecessary transfers of the nicotine. Therefore, the demethylation was carried out on the nicotine dipicrate.

Twenty to 60 mg. of nicotine dipicrate, 45 mg. ammonium iodide, 2 drops 5 percent gold chloride and 3 ml. of hydriodic acid (sp. gr. of 1.7) were placed in the reaction vessel and the vessel attached to the demethylation train (2). The gas washing vessel contained 1.5 ml. cadmium sulfate-sodium thiosulfate solution which removed iodine and hydroiodic acid from the gas stream. The delivery tube was placed below the surface of a 5 percent solution of triethylamine in ethanol in the receiving tube which was cooled to -70° C. in a methyl cellosolve-solid carbon dioxide bath. Nitrogen gas was passed slowly through the demethylation train during the entire operation.

The reaction flask was placed in a copper oxide bath and heated to 200° C. in 20 to 25 minutes. The temperature was then raised slowly to 350 to 360° C. and allowed to remain at this temperature for 45 minutes. The heating element was removed and sweeping with nitrogen gas was continued for 15 minutes. The delivery tube was rinsed with ethanol and the rinsings placed in the receiving flask.

The solution in the receiving flask was mixed, stoppered and allowed to stand for 12 hours. After standing, the solution was evaporated to a small volume on a steam bath with a slow stream of air directed on the liquid surface. The remaining alcohol and triethylamine were removed in a vacuum desiccator. The resulting

methyltriethylammonium iodide, a white crystalline solid, was transferred quantitatively to a tared aluminum planchet for determination of weight and C^{14} content.

Results

The data shown in Table II illustrate that demethylation of nicotine dipicrate is feasible and yields are similar to those obtained from nicotine hydrochloride (2). The reliability of this procedure is illustrated further in Table III. Radioactive nicotine containing C^{14} only in the methyl group (14) was demethylated and the specific activity of the methyltriethylammonium iodide was essentially equal to the specific activity of the original nicotine dipicrate.

TABLE II

DEMETHYLATION OF NICOTINE DIPICRATE

Nicotine dipicrate mg.	Methyltriethyl-ammonium iodide mg.	Percent Yield
53.5	17.8	85
27.9	7.6	70
20.0	6.3	80

TABLE III

RECOVERY OF C^{14} FROM METHYL-C^{14}-NICOTINE DIPICRATE

Maximum Specific Activity (Counts per minute per millimole)	
Nicotine dipicrate	Methyltriethyl-ammonium iodide
4.0×10^5	3.9×10^5
2.5×10^5	2.5×10^5

A sample of the calculations is shown in the appendix.

Determination of Radioactivity

All counts were made with a Nuclear-Chicago Model 192X scaler (Nuclear Instruments and Chemical Corporation, Chicago 10, Illinois) and a Tracerlab Model SC-16 proportional flow counter (Tracerlab, Inc., Boston 10, Massachusetts). The counting system was 40 percent efficient as determined by a National Bureau of Standards $Na_2C^{14}O_3$ sample.

The substances to be counted, with the exception of D-ribose-1-C^{14}, were ground in a small agate mortar or dissolved in ethanol and placed in aluminum planchets. The material was distributed in the planchets and the surface made as smooth as possible before counting. The activity of the compounds was corrected to "infinite thickness" by reference to a $BaC^{14}O_3$ self absorption curve. D-ribose-1-C^{14} was counted as an infinitely thin layer.

Radioactivity on chromatograms was located and estimated with a Forro chromatograph scanner (Forro Scientific Company, Evanston, Illinois)

coupled with a Nuclear-Chicago Model 1620A ratemeter and a Model AW Esterline-Angus graphic ammeter.

The nicotine dipicrate and methyltriethylammonium iodide samples were corrected to 100 percent efficiency of the counting system. The specific activity of the other compounds were reported without correcting for the efficiency of the counting system.

Purity of D-Ribose-1-C^{14}

D-ribose-1-C^{14} (purchased from Nuclear Instruments and Chemical Corporation, Chicago 10, Illinois) was co-chromatographed with authentic D-ribose with water saturated phenol and propionic acid-butanol-water solvents (54). The R_f values of the pentose corresponded with the radioactive area on the chromatograms. No other radioactive area was detected with the chromatograph strip counter.

Uptake of D-Ribose from Nutrient Solution

Several studies in this laboratory (2,9,12,13,14,35) have shown that tobacco plants can absorb various nutrients through their root systems from nutrient solution. Before administration of D-ribose-1-C^{14}, it was necessary to ascertain the absorption rate of ribose and, also, to determine if microorganisms altered the ribose molecule before absorption occurred.

A colorimetric method of analysis was used to estimate ribose in the nutrient solutions (55). A compound with an absorption maximum at 660 mμ is formed by a reaction of pentoses, ferric chloride and orcinol

in strong acid solution. Three ml. of an aqueous solution containing between 4-40 ug. of pentose was added to 3 ml. of 1 percent orcinol in 0.1 percent ferric chloride dissolved in concentrated hydrochloric acid. The mixture was heated for 30 minutes in a boiling water bath, cooled to room temperature and the optical density determined with a Beckman Model B spectrophotometer. A standard reference curve was prepared using known quantities of D-ribose. Since the nutrient solution contained several inorganic compounds that might interfere during the determination, the solutions were treated with 0.1 gm. Dowex 50 and 0.1 gm. Dowex 1 resins (Dow Chemical Company, Midland, Michigan) before determining the residual ribose.

Fifty ml. of nutrient solution, 3 drops of detergent germacide and 4 mg. of D-ribose were placed in 12 flasks. Tobacco plants were placed in 4 flasks, 4 were inoculated with six root fragments 1 cm. in length and the four remaining flasks received no plant material. After 36 hours the nutrient solutions were deionized and the D-ribose was determined.

Results

No loss of ribose was detected in the control solutions and solutions containing root fragments. The nutrient solution in which plants were grown contained no ribose. These results indicate a complete and rapid absorption of ribose from the nutrient solutions and no detectable microbial destruction occurred under these conditions.

Metabolism of D-Ribose-1-C^{14} by Tobacco Plants

Pentose phsophates participate in most metabolic reactions of the pentoses in plants. The chemical literature contains no report concerning the presence of a pentose kinase in plants. Therefore, it was desirable to establish whether ribose was metabolized after being absorbed by tobacco plants. Also, it was of interest to determine the general distribution of C^{14} in plant substances after feeding D-ribose-1-C^{14} and whether an added carbon source in the nutrient solution would decrease oxidation of the ribose by the plants.

Two tobacco plants were prepared for hydroponic administration of ribose as previously described. Two mg. of D-ribose-1-C^{14} (1×10^6 counts per minute) was placed in the nutrient solution of each plant and 10 mg. of D-glucose was added to the nutrient solution of one plant. The plants were enclosed in separate glass containers and carbon dioxide free air was passed through the containers. The respiratory carbon dioxide in the exhaust air stream was collected in a gas washing bottle containing 5 ml. of 0.1 N NaOH. The plants were allowed to metabolize the ribose for 36 hours with light during the first and last 12 hour periods.

Following the feeding period the plants were removed from the flasks, dried and ground with a mortar and pestle. The radioactivity of the plant material was determined on a flow counter and corrected for self absorption. The C^{14} remaining in the nutrient solution was determined by placing a small amount of the liquid in a planchet and

the water evaporated with the aid of a heat lamp. The respiratory carbon dioxide was collected as barium carbonate and counted as such.

After determining the radioactivity in the dry plant material, starch was isolated by the method of Hassid et al. (56). This method employs an acid alcohol and a water extraction. The insoluble residue from the starch preparation was also analyzed for radioactivity. The residue consisted of cell wall constituents and part of the plant proteins.

Results

The distribution of radioactivity after feeding the plants is shown in Table IV. About 85 percent of the radioactivity was recovered in these experiments. This discrepancy might be due to backscattering of the beta particle during the counting of the ribose-1-C^{14}. D-ribose-1-C^{14} was counted as an infinitely thin layer in aluminum planchets. When counting samples at "infinite thinness," many particles are reflected into the sensitive volume of the counting chamber. This effect causes the observed number of counts to be higher than the actual value. All the other plant fractions were counted under conditions where backscattering could not occur and were corrected to "infinite thickness" with a self absorption curve.

Tolbert and Gailey (36) have shown that carbon dioxide fixation was stimulated when leaves were sprayed with a ribose solution. This indicated that ribose could be phosphorylated and utilized by plants. These studies show that ribose can be metabolized by plants.

TABLE IV

LOCATION OF RADIOACTIVITY AFTER FEEDING TOBACCO PLANTS D-RIBOSE-1-C^{14}

Compounds Fed	Percent C^{14} Recovered				
	Respiratory Carbon dioxide	Nutrient Solution	Plant Material	Starch	"Cell wall Substances"
D-Ribose-1-C^{14}	23	11	66	0.4	39
D-Ribose-1-C^{14} plus Glucose	18	45	37	0.2	25

Ribokinase has been purified from animal tissue (57) and is probably present in plant tissue also. Recently, Niesh (58) has shown that wheat plants metabolize arabinose, presumably, by synthesis of the pentose-5-phosphate initially.

A very striking result was that about one-fifth of the C^{14} appears as respiratory carbon dioxide. The plants apparently absorb and oxidize the ribose very rapidly. The metabolic rate further emphasized by the incorporation of a large quantity of the C^{14} into the insoluble constituents. Starch contained only a small fraction of the activity. However the quantity of starch present was very small as would be expected when plants are grown in an atmosphere limiting in carbon dioxide.

The quantity of C^{14} remaining in the nutrient solution of the plant fed ribose only was probably a result of root fragments and material excreted from the plants as it was shown previously that ribose is absorbed from the nutrient solution in 36 hours. The nutrient

solution in which glucose was added contained almost one-half the radioactivity originally fed. Although no definite conclusions may be gained with the limited information obtained, it appears that the rate of ribose absorption was considerably slower in the presence of glucose. Glucose had no sparing effect on the oxidation of ribose as the quantities of C^{14} respired in the two experiments were similar.

Methylation Studies with D-Ribose-1-C^{14}

These experiments were undertaken to study the synthesis of methyl groups from ribose in plants. The formation of glycolic acid from pentose phosphates and the subsequent conversion of the alpha carbon of glycolic acid into methyl groups has been postulated to be a pathway for synthesis of the one carbon unit in plants. The methyl group of nicotine was shown to be synthesized from the alpha carbon of glycolic acid by Byerrum *et al.* (13). If this pathway does exist the one carbon of ribose would be incorporated into the methyl group of nicotine in tobacco plants. Wilson and Calvin (29) have shown that plants growing in the absence of carbon dioxide produce greater amounts of glycolic acid. In these studies the plants were grown in an atmosphere in which carbon dioxide concentration was minimized in order to increase the concentration of glycolic acid.

The plants were prepared as described previously. Two mg. of D-ribose-1-C^{14} with known activity and 10 mg. D-glucose was dissolved in the nutrient solutions. The plants were placed in a 10 inch desiccator

and carbon dioxide free air passed through the desiccator at the rate of 0.2 liters per minute. Following the growing period the plants were harvested and nicotine isolated and demethylated as described in previous sections of this report.

Results

Nicotine isolated from plants fed D-ribose-1-C^{14} was radioactive. The results, presented in Table V, show that only a very small part of the C^{14} was incorporated into the methyl group of nicotine. The distribution of C^{14} indicates that a rapid and, possibly, a specific synthesis of the nicotine ring system from ribose occurs, and that the rate of synthesis of methyl groups is relatively slow.

Formation of Glycine and Serine in Detached Tobacco Plant Leaves

Previous work in this laboratory has shown that the alpha carbon of glycine and the beta carbon of serine may be converted into the methyl group of nicotine in tobacco plants (12,14). It has been postulated that these amino acids might be formed from a two carbon compound (glycolaldehyde) which is formed in the photosynthetic cycle (19,29). Tolbert and Cohen (18) and Schou et al. (37) have shown that glycine and serine may be formed from glycolic acid in plants. Thus, the formation of glycine, serine or glycolic acid from the photosynthetic cycle would establish a pathway in plants for the synthesis of methyl groups from carbon dioxide.

TABLE V

LOCATION OF RADIOACTIVITY IN NICOTINE FROM TOBACCO PLANTS FED D-RIBOSE-1-C^{14}

Number of Plants	Activity Fed per Plant (cpm)	Feeding Period (days)	Maximum Specific Activity (Counts per Minute per Millimole)		Percent C^{14} in Methyl Group
			Nicotine dipicrate	Methyltriethyl-ammonium iodide	
10	1.0×10^7	7	4.2×10^3	2.8×10^2 2.4×10	6.7
10	1.0×10^7	7	3.2×10^3	2.4×10^2	7.5
5	2.6×10^7	3	19.0×10^3	12.2×10^2	6.3
5	2.6×10^7	2	14.0×10^3	4.4×10^2	3.1

A sample calculation of specific activity is shown in the appendix.

The extremely rapid metabolism of ribose by tobacco plants limits the value of the hydroponic method of feeding plants for studying methyl group synthesis. The absorption of ribose from the nutrient solution was very slow as compared to the metabolic transformations of the pentose and a rapid randomization of the C^{14} occurred, that is, the C^{14} is distributed to all carbons of the ribose. The initial reactions of D-ribose-1-C^{14} are masked by similar reactions of uniformly labeled ribose. Thus, the compounds formed from ribose would be nearly uniformly labeled. A method was desired in which the pentose could be introduced rapidly into tobacco plants and the plants allowed to metabolize for short periods. The vacuum infiltration method was employed in these experiments (59).

Vacuum Infiltration of D-Ribose-1-C^{14} into Detached Leaves

Leaves from three month old tobacco plants were immersed in an aqueous solution containing 2 mg. D-ribose-1-C^{14} (8×10^5 counts per minute) per ml. The pressure above the solution was reduced to 30 mm. mercury to remove the air from the intercellular spaces of the leaves. After one minute, air was allowed to enter the chamber and the solution surrounding the leaves was drawn into the intercellular spaces. The leaves were weighed before and after infiltration to determine the weight of infiltrated D-ribose-1-C^{14}. The leaves weighed approximately 0.6 g. each and the amount of infiltrated solution was about 50 percent of the weight of the original leaf. The stems of the leaves were placed between two pieces of moistened filter paper during the period for metabolism.

Several leaves were placed in a light proof container to study metabolism of pentoses in the absence of light and several were illuminated with fluorescent tubes with a light intensity of about 200 footcandles. Following a three hour period for metabolism the leaves were harvested, cut into small pieces and placed in boiling 80 percent ethanol. The mixture was cooled and homogenized in a ground glass, motor driven homogenizer. The homogenate was filtered with Whatman No. 2 filter paper to remove cell debris and insoluble substances. The filtrate was diluted to 10 ml. with water and 0.8 mg. carrier glycine and serine added.

The radioactivity of free ribose remaining in the leaves was estimated by chromatographing an aliquot of the extract and determining the C^{14} in the ribose area with a chromatograph strip counter. The resolving solvent was t-amyl alcohol and acetate buffer, pH 5.6, (60) and analine-phthalic acid reagent (61) was used to identify the ribose on the chromatogram.

Isolation and Purification of Serine and Glycine

Glycine and serine were isolated from leaf extracts by one-dimensional paper chromatographic methods. The resolving solvents were employed in the following order:

(1) t-Amyl alcohol saturated with 0.1 M sodium acetate buffer, pH 5.6 (60).

(2) Phenol saturated with an aqueous solution of 3.7 percent sodium dihydrogen phosphate and 6.3 percent sodium citrate (62).

(3) Pyridine and water (65:35) (62).

Phenol and salts were C. P. grade and the organic solvents were redistilled before using.

The areas of the chromatograms containing the amino acids were eluted with water and placed on another sheet of filter paper for resolution with the succeeding solvent. Authentic glycine and serine was placed on all chromatograms to identify the amino acid areas following resolution.

Approximately 1 ml. of the 80 percent ethanol extract was applied in contiguous droplets to an area of 1 x 20 cm. on a sheet (30 x 40 cm.) of Whatman No. 1 filter paper. The extract was applied on a line 5 cm. from the narrower edge of the chromatogram. The solutions were applied with micropipettes and a stream of warm air was used to facilitate evaporation of the solvent. The chromatograms were stapled in the form of a cylinder in such a manner that the edges did not touch. This self supporting cylindrical chromatogram was placed upright in a 15 x 45 cm. glass jar that contained 50 ml. of the resolving solvent. The jars were sealed and the solvent allowed to ascend until the solvent front was 8-10 cm. from the top of the filter paper. Resolution was complete in 20 to 48 hours at room temperature.

The chromatograms were removed from the jars and placed in a forced draft oven and dried for one hour at 200° F. The amino acid areas were located by cutting the strips from the chromatogram that contained known samples of glycine and serine and spraying with ninhydrin solution (62). The corresponding areas that contained glycine and serine from the plant extracts were removed from the chromatograms.

Amino acids were eluted from the paper with water. A filter paper wick was fastened to one end of the paper strip and the end of the wick was immersed in water. The elution was accomplished by capillary movement of water through the filter paper. The elution was continued until 2 ml. of eluate was collected. Glycine and serine solutions were placed on another filter paper sheet for purification with the succeeding solvent. The eluate containing glycine and serine from the final chromatogram was evaporated to 1 ml. on the steam bath. Forty mg. serine or 20 mg. glycine was dissolved in the solutions as additional carrier.

The purity was established by recrystallizing the amino acids until their specific activity was constant. Glycine and serine were obtained in crystalline form by adding 0.1 ml. pyridine and sufficient acetone to cause precipitation in 1 ml. of aqueous amino acid solution. The precipitate was collected on a filter and transferred to a planchet to determine the specific activity. Two recrystallizations were sufficient to yield essentially pure amino acids.

Degradation of Glycine

Glycine was degraded by the ninhydrin method (63) which is summarized below:

Ten mg. of glycine was dissolved in 5 ml. of water and the solution placed in one side of a two compartment reaction vessel.

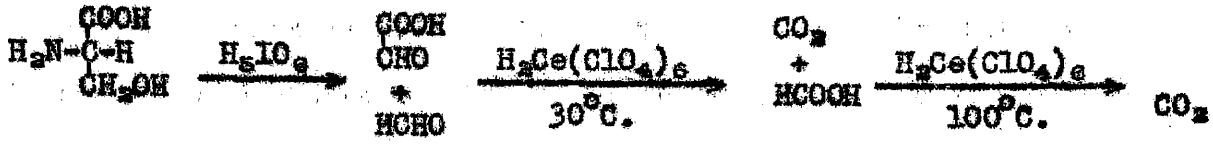
One hundred mg. citric acid and 150 mg. ninhydrin were placed in the other compartment. The vessel was swept with nitrogen gas for five minutes and a gas washing bottle containing 5 ml. of 0.1 M sodium hydroxide was attached. The reactants were mixed by tilting the vessel in such a manner that the glycine solution flowed into the ninhydrin and citric acid mixture. The reaction was allowed to proceed at 100° C. for 25 minutes. Nitrogen gas was passed slowly through the system during the reaction period.

When the reaction was complete, the carbon dioxide collected in the gas washing bottle was precipitated as barium carbonate. The yield of carbon dioxide from the carboxyl car on was 80-85 percent of the theoretical value.

The formaldehyde remaining in the reaction vessel was distilled in vacuo. Five ml. of water was added to the residue in the reaction vessel and the solution concentrated to dryness again. The vacuum distillations were performed at 50° C. Three ml. of (.8 percent dimedon (5,5-dimethylcyclohexanedione-1,3) in 95 percent ethanol was added to the combined distillate. The formaldehyde and dimedon solution was adjusted to pH 5.6 with acetic acid and allowed to stand for 24 hours. The dimedon derivative of the formaldehyde was collected on a filter and washed with 5 ml. of cold water containing one drop of 1 M acetic acid. The melting point was 188-189° C. The yield of formaldehyde from the alpha carbon of glycine was 25-35 percent of the theoretical yield.

Degradation of Serine

Serine was degraded by modifying the procedure proposed by Aronoff (64) as illustrated in the equation below:



The oxidation of serine with periodic acid was reported by Nicolet and Shinn (64) to proceed slowly and usually to take 10 to 24 hours to complete. Also, the oxidation of glyoxylic acid, a product formed from the initial oxidation of serine, was reported to be insignificant during this reaction period (40,64). It was observed in this study that serine was oxidized rapidly with periodic acid. The reaction was complete in about 30 minutes at room temperature. When two equivalents of periodic acid were used, the excess periodate oxidized the glyoxylic acid rapidly. Both oxidations were complete in one hour.

Twenty mg. serine and 39.1 mg. periodic acid were dissolved in 5 ml. of water and the reaction mixture allowed to stand for one hour at room temperature (about 25° C.). The solution containing formaldehyde and glyoxylic acid was adjusted to pH 8.5 with sodium hydroxide and the formaldehyde removed by two successive vacuum distillations. The formaldehyde was isolated as described in the preceding section. The reaction flask containing the residual sodium glyoxylate was swept with nitrogen gas for five minutes and a gas washing bottle containing 0.1 N sodium hydroxide was attached. Nitrogen gas was passed slowly through the system during the following procedures. Five ml. of 0.5 M

perchloratocerate solution (G. F. Smith Company, Columbus, Ohio) was added through a side arm. The reaction was allowed to continue for 15 minutes at 30°C. Following the reaction period the gas washing bottle was recharged with 5 ml. 0.1 N sodium hydroxide and the reaction temperature increased to 100° C. for five minutes. The reaction mixture was allowed to cool and sweeping with nitrogen gas continued for 10 minutes. The carbon dioxide collected in the gas washing bottle was precipitated with barium chloride solution and the barium carbonate collected on a filter. The dimedon derivative of formaldehyde and barium carbonate samples were weighed and the radioactivity determined.

The yield of the formaldehyde from the beta carbon of serine was 60-70 percent of the theoretical value and barium carbonate from the alpha and carboxyl carbons was obtained in 70-80 percent of theoretical yields.

Results

The data in Table VI show that ribose was absorbed into the cells of the leaves from the intercellular spaces and rapidly metabolized during the three hour period. The metabolic rate decreased in the absence of light. Approximately 37 percent of the D-ribose-1-C^{14} remained in the leaves which were not illuminated whereas only 18 percent was recovered from the illuminated leaves.

Glycine and serine isolated from the illuminated leaves was radioactive. The glycine from leaves fed in the absence of light did not contain detectable quantities of C^{14}, however, the serine was radioactive.

TABLE VI

METABOLISM OF D-RIBOSE-1-C^{14} BY DETACHED LEAVES

Experiment	Number of Leaves	C^{14} Infiltrated into Leaves (cpm)	C^{14} Recovered as Free Ribose (cpm)
Dark	4 (2.53 g.)	9.2×10^5	3.4×10^5
Light	4 (2.78 g.)	10.1×10^5	1.8×10^5

Each figure is the average of two experiments. Less than five percent variation in individual experiments occurred.

The location of the C^{14} in serine from the light and dark experiments is similar although the serine from illuminated leaves contained more total activity. Serine was isolated from 5.2 ml. of the illuminated leaf extract and 4.4 ml. of the extract from leaves grown in the dark. Also, a greater quantity of ribose was metabolized in the leaves which were illuminated. When this information is considered, it can be seen that about the same percent of C^{14} from the ribose metabolized was incorporated into serine in either light or dark. These results are interpreted in greater detail in the Discussion part of this thesis.

TABLE VII

DISTRIBUTION OF C^{14} IN SERINE

Experiment	Maximum Specific Activity (counts per minute per millimole x 10^{-2}) Serine (a)	Barium carbonate (carboxyl carbon) (b)	Barium carbonate (alpha carbon) (c)	Dimedon Derivative (beta carbon) (d)	Percent C^{14} Recovered $\left(\frac{b+c+d}{a} \times 100\right)$
Dark	2.78	0.16	1.02	1.45	95
Light	5.64	0.60	2.15	2.45	92

TABLE VIII

DISTRIBUTION OF C^{14} IN GLYCINE

Experiment	Maximum Specific Activity (counts per minute per millimole x 10^{-2}) Glycine (a)	Barium carbonate (carboxyl carbon) (b)	Dimedon Derivative (alpha carbon) (c)	Percent C^{14} Recovered $\left(\frac{b+c}{a} \times 100\right)$
Dark	0.0	----	----	----
Light	3.84	1.12	2.59	97

DISCUSSION

DISCUSSION

Synthesis of Nicotine from D-Ribose

Nicotine synthesized in tobacco plants which were fed D-ribose-1-C^{14} was radioactive. The methyl group contained only a small part of the C^{14} of the nicotine molecule. About seven percent of the C^{14} was in the methyl group of nicotine from plants which were harvested three and seven days after administration of the radioactive compound. When the plants were harvested two days after administration of the ribose, the nicotine methyl group contained three percent of the C^{14}. The shorter metabolism periods did not result in a large decrease in the incorporation of the C^{14} into the nicotine molecule.

The transfer of methyl groups to nicotine in tobacco plants appears to be a relatively slow metabolic reaction (2,65). Leete (65) has observed that the radioactivity of nicotine increased linearly up to five days when tobacco plants were fed methyl-C^{14}-methionine. No further increase in C^{14} content was noted when the period for metabolism was extended to three weeks. In contrast, the metabolic transformations of pentoses in plants appear to be extremely rapid. Calvin and co-workers (28,29,39,66) have studied $C^{14}O_2$ incorporated into the pentose phosphates and related metabolites. Changes in C^{14} concentration were detected after a period of less than one second. It was noted in the present study that about twenty percent of the C^{14}, from D-ribose-1-C^{14} administered to plants, was respired in 36 hours and a large part of

the C^{14} was incorporated into the insoluble cell wall substances. The rapid rate and great variety of the metabolic reactions of pentoses and their phosphate esters precludes the possibility that a large quantity of the pentose would be utilized for methyl group synthesis.

Several pathways have been suggested for the formation of one carbon units from pentose phosphates. The initial step in these postulated metabolic pathways is the synthesis of a two or three carbon compound which then is converted to glycine, serine or glycolic acid. Glycine, serine and glycolic acid are interrelated in plant metabolism and appear to be synthesized from a common precursor (18,37). The dilution of C^{14} when serine-3-C^{14} was converted to nicotine in tobacco plants was about 2000. The alpha carbons of glycine and glycolic acid are converted into the nicotine methyl group to a similar extent. Assuming ribose was converted to glycine, serine, or glycolic acid an additional large dilution of the C^{14} would be expected. Thus, the dilution of C^{14} in converting D-ribose-1-C^{14} to the nicotine methyl group would be 2000 times the dilution in converting ribose to serine, glycine and/or glycolic acid.

The C^{14} from D-ribose appears to be incorporated into nicotine at a rate greater than several other C^{14} labeled compounds which were tested. Dewey (67) and Lamberts (68) have studied incorporation of ornithine and glutamic acid into the pyrrolidine ring of nicotine. The C^{14} was not rapidly incorporated into nicotine when tobacco plants were fed the C^{14}-labeled amino acids under conditions similar to those used in the present studies.

The C^{14} content of nicotine from plants fed D-ribose-1-C^{14} was similar in experiments in which plants were allowed to metabolize two, three, and seven days following the administration of labeled pentose. These results indicate a rapid and possibly specific synthesis of the nicotine ring system. Since the rate of incorporation of C^{14} into the pyrrolidine ring was slow when an immediate precursor, such as glutamic acid, was fed, the C^{14} from D-ribose-1-C^{14} may have been incorporated into the pyridine ring of the nicotine. Although these observations are without experimental proof, the suggestions appear tenable.

Frontera-Aymat (35) administered pyruvic acid-3-C^{14} to tobacco plants and found the nicotine to be radioactive. The methyl group contained about six percent of the total activity of the nicotine. Lamberts (68) has shown that glutamic acid-2-C^{14} is incorporated into nicotine. About ten percent of the activity was in the pyridine ring.

All radioactive compounds administered to plants and incorporated in the nicotine ring system were related to intermediates in glycolysis or the tricarboxylic acid cycle. The results suggest that a compound such as acetate or possibly a pentose derivative can be converted rapidly into nicotine in the tobacco plant.

Very little information is available concerning the biosynthesis of the nicotine molecule. Ornithine and glutamic acid have been shown to form the pyrrolidine ring of nicotine (67,68). The synthesis of the pyridine ring is still unsolved. The formation of nicotinic acid in animals and bacteria has been shown to arise in part from tryptophan. However, the formation of the pyridine ring from tryptophan catabolism

does not appear to be an important pathway in plant metabolism (69). These observations may be of importance in the elucidation of the biosynthesis of the pyridine ring of nicotine.

Synthesis of Serine and Glycine from D-Ribose in Detached Leaves

Several metabolic pathways for serine and glycine synthesis from D-ribose-1-C^{14} are shown in Figure 1. The colored dots adjacent to the carbon atoms denote the major forward metabolic pathway for these atoms. Reactions which have not been sufficiently established are indicated by dotted lines.

The C^{14} distribution in serine and glycine synthesized from D-ribose-1-C^{14} cannot be accounted for by any single known metabolic pathway. The C^{14} distribution suggests that at least two metabolic pathways are utilized for serine synthesis. One possible explanation of the data would be for a portion of the serine to be synthesized from 3-phosphoglyceric acid (reaction E, Figure 1). Glycine and additional serine could be synthesized from glyoxylic acid produced in the glyoxylate cycle (reaction F, Figure 1). Endogenous $C^{14}O_2$ is assumed to be fixed primarily by condensation with phosphoenolpyruvic acid (reaction D, Figure 1).

Glycine, glycolic acid, or glyoxylic acid synthesized from D-ribose-1-C^{14} would be labeled in the alpha carbon atoms. Synthesis of serine and glycine from glycolic acid has been demonstrated in plants (18,37). The C^{14} content of the beta carbon would be less than, or at best equal to, the C^{14} content of the alpha carbon of serine if it were

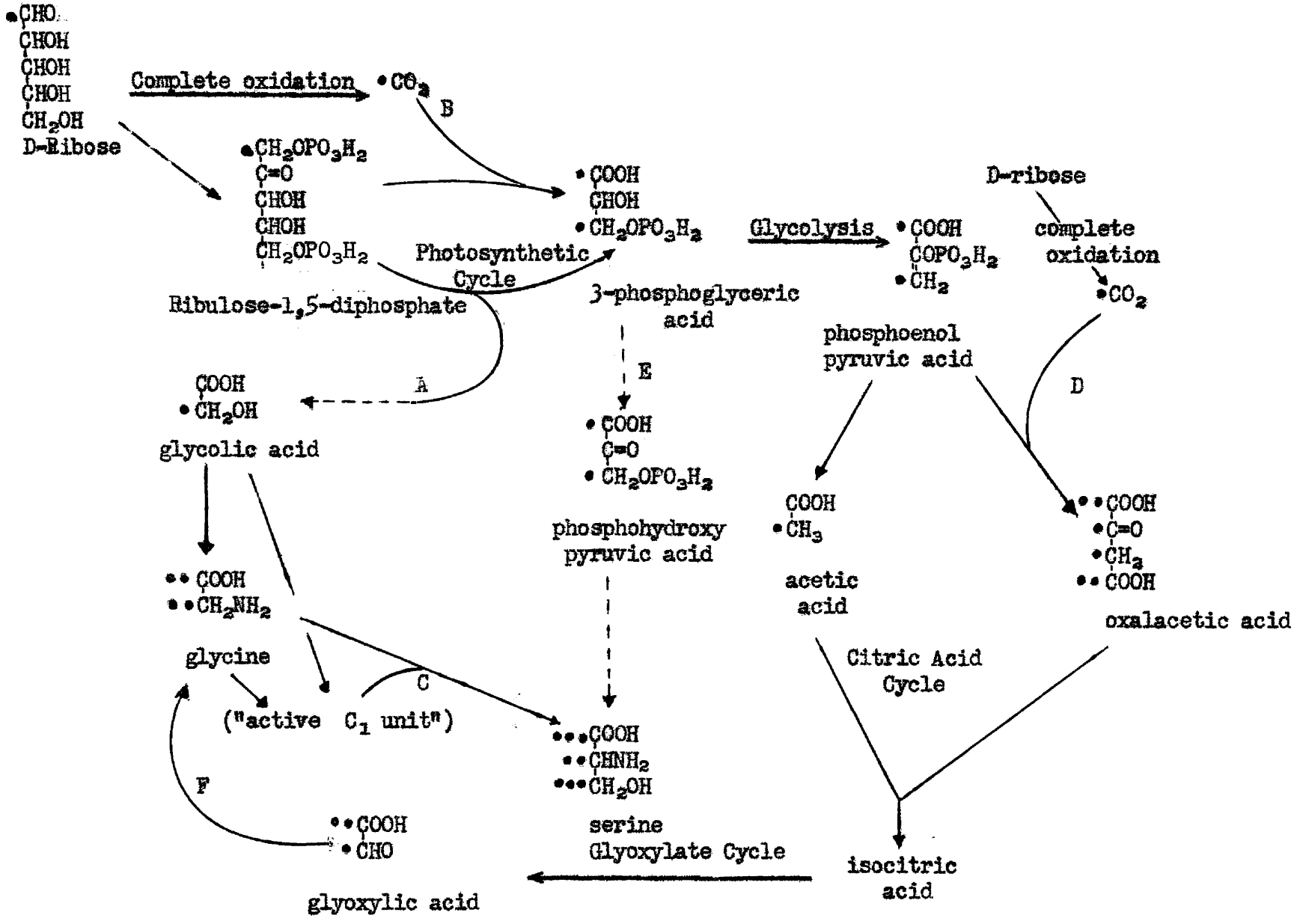


Figure 1. Biosynthesis of glycine and serine in plants.

synthesized only from glycolic acid or glycine since dilution of the one carbon unit would be expected (reaction C, Figure 1) (18). However, partial randomization of the C^{14} in the alpha and beta carbons of serine does indicate that serine was synthesized primarily from a two carbon precursor.

Glycine has been shown to be synthesized from glycolic or glyoxylic acids in plants (18,37). The synthesis of glycine from serine, as was postulated to occur in animal metabolism (33), appears to be of minor significance in tobacco plants. The specific activities of the carboxyl and alpha carbons of glycine and serine were not identical as would be expected if glycine were synthesized from serine.

The two carbon precursor of glycine and serine is believed to be produced in the glyoxylate cycle. This postulate is based on two lines of reasoning. First, the C^{14} of the one carbon of ribose cannot be incorporated into the two, three, or four carbons of pentose phosphates by known reactions of glycolysis or the photosynthetic cycle. Therefore, C^{14} in the carboxyl carbon of glycine or serine must originate from endogenous $C^{14}O_2$. The reaction of $C^{14}O_2$ and ribulose diphosphate (reaction B, Figure 1) would yield only 3-phosphoglyceric acid-1-C^{14}. Serine synthesized from 3-phosphoglyceric acid would contain a greater percentage of the C^{14} in the carboxyl carbon than glycine synthesized from a two carbon precursor. If $C^{14}O_2$ was metabolized by fixation with phosphoenolpyruvic acid, glyoxylic acid synthesized from it, would contain C^{14} in the carboxyl carbon. Glycine and serine formed from

the glyoxylic acid then would contain equal quantities of C^{14} in the carboxyl carbon.

The dilution of C^{14} in the serine carboxyl carbon probably occurred by a direct conversion of 3-phosphoglyceric acid to serine. The fraction of serine formed from 3-phosphoglyceric acid which is labeled only in the beta carbon would increase the specific activity of the serine beta carbon. The fixation reaction of carbon dioxide and phosphoenolpyruvic acid is firmly established in plant metabolism (70) and appears to be particularly significant during dark carbon dioxide fixation (71).

Secondly, the distribution of C^{14} in serine from the dark metabolism of D-ribose-1-C^{14} also provides support for the postulate that the two carbon precursor of glycine and serine is synthesized in the glyoxylate cycle. The percentage of C^{14} in the beta carbon of serine was greater and the C^{14} content of the carboxyl carbon was less than the corresponding carbons of serine synthesized during light metabolism. These data indicate that serine synthesized from a two carbon precursor contributes a smaller part of the total serine synthesized. This result is consistent with previous observations on light and dark metabolism. Stutz and Burris (72) and Benson and Calvin (21) noted that glycolic acid disappeared from plant tissues when the plants were placed in the dark. Other reports indicate that glycine is not synthesized or synthesized very slowly during dark metabolism in plants (71,73). Glycine-C^{14} was not detected when D-ribose-1-C^{14} was metabolized in the non-illuminated leaves. Therefore, only a small quantity of serine could

have been synthesized from a two carbon precursor. The C^{14} distribution of serine formed in leaves during dark metabolism of D-ribose-1-C^{14} would be expected to contain C^{14} primarily in the beta carbon and only a very small part of the C^{14} would appear in the carboxyl carbon. It may be seen that data obtained from degradation of serine supports this hypothesis.

Unfortunately, the specific activity of glycine and serine from light metabolism could not be calculated since the total amounts of glycine and serine in the intact plants was not determined. If the specific activity of the carboxyl and alpha carbons of serine were greater than the corresponding glycine carbon atoms the hypothesis that serine was not synthesized exclusively from glycine or other two carbon precursors would be established.

The mechanisms for glycine and serine synthesis postulated from the results of this study are consistent with data reported from other laboratories. Newburgh and Burris (34) administered pyruvic acid-2-C^{14} to plants and isolated glycine containing essentially all of the C^{14} in the carboxyl carbon atom. When plants were allowed to photosynthesize $C^{14}O_2$ for 15 and 50 seconds, C^{14} was incorporated into the carboxyl carbon of serine and to a lesser extent into the alpha and beta carbons of serine and both carbons of glycine (17). Nyc and Zabin (74) administered pyruvic acid-3-C^{14} to rats and the labeling distribution in glycine and serine was similar to that found in this study. Data from reports in which $HC^{14}OOK$, acetate-1-C^{14}, acetate-2-C^{14} and

pyruvic acid-2-C^{14} were administered to plants, animals, or micro-organisms also support this mechanism for glycine and serine synthesis (73,74,75).

The conversion of D-ribose-1-C^{14} to glycine labeled in the alpha carbon and serine labeled in the beta carbon demonstrated one pathway by which methyl groups may be synthesized in plants. It was stated previously that glycine-2-C^{14} (12) and serine-3-C^{14} (14) were metabolized by plants and the C^{14} incorporated into the methyl group of nicotine. It was suggested that the majority of glycine and serine was synthesized from glyoxylic acid which was produced in the glyoxylate cycle. Additional experiments should be performed to further establish the biogenesis of these amino acids. It would be of interest to administer radioactive isocitric acid to plants and study the labeling distribution in glycine, serine and nicotine.

SUMMARY

SUMMARY

1. Tobacco plants absorbed D-ribose-1-C^{14} from an aqueous nutrient solution. About 20 percent of the C^{14} was respired as $C^{14}O_2$ during a 36 hour growing period. Approximately one-third of the C^{14} was located in the water- and 80 percent ethanol-insoluble constituents.

2. Nicotine isolated from tobacco plants fed D-ribose-1-C^{14} was radioactive. The N-methyl group of nicotine contained only a small part of the C^{14}. The rate of incorporation of C^{14} into the nicotine molecule suggested that a specific incorporation of a glycolysis intermediate into the pyridine ring occurred.

3. Tobacco plants leaves were vacuum infiltrated with D-ribose-C^{14} solution. The leaves which were allowed to metabolize in the dark synthesized serine-C^{14} but no radioactive glycine. Illuminated leaves synthesized radioactive glycine and serine.

4. Degradation of glycine and serine synthesized from D-ribose-1-C^{14} showed the C^{14} to be located primarily in the alpha and beta carbons of serine and the alpha carbon of glycine. A mechanism for glycine and serine biogenesis in plants was proposed and discussed.

5. The isolation of glycine and serine containing C^{14} in the alpha and beta carbons showed one possible pathway for synthesis of methyl groups from the photosynthetic cycle.

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LITERATURE CITED

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APPENDIX

APPENDIX

The formula used in correcting the observed count to zero sample thickness was:

$$A_m = \frac{C_o \cdot M}{W \cdot b}$$

where A_m = maximum specific activity (counts/minute/millimole)

C_o = observed counts (counts/minute)

M = molecular weight of compound

W = weight of sample counted

b = fraction of maximum activity at the sample thickness used (T)--obtained from self-absorption curve.

Sample calculation:

Nicotine dipicrate --- C_o = 161.2 c.p.m., W = 29.0 mg., M = 620

T = 10.2 mg./cm.^{2}, b = 0.440.

$$A_m = \frac{161.2 \cdot 620}{0.330 \times 29.0} = 7.83 \times 10^3 \text{ c.p.m./millimole}$$

Efficiency of counting system = 40%

Correction of specific activity to 100%:

$$\frac{7.83 \times 10^3}{.40} = 1.96 \times 10^4 \text{ c.p.m./millimole}$$