BIOSYNTHESIS OF DEOXYRIBOSE IN WHEAT EMBRYOS

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ABSTRACT

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Biosynthesis of Deoxyribose in Wheat Embryos

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The biosynthesis of deoxyribose in wheat embryos was studied using isotope tracer techniques and chemical degradation procedures.

When glucose -6-C^{14} and glucose-1-C^{14} were supplied to wheat embryos, the highest amount of radioactivity was found on carbon five of deoxyribose. Embryo feeding with glucose-2-C^{14}, on the otherhand, resulted in a high amount of radioactivity on carbon four of deoxyribose and considerable radioactivity on carbon five.

On the basis of these results, it is suggested that a combination of two pathways is involved in deoxyribose biosynthesis. In the oxidative pathway, carbon two through carbon six respectively of glucose furnish carbon one through carbon five respectively of ribose, while carbon one of glucose is lost as carbon dioxide. The non-oxidative pathway involves sequential reactions catalyzed by transaldolase and transketolase.

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INTRODUCTION

Since nucleic acids have been found to have such important roles in living organisms, the biosyntheses of nucleic acids and their sugar moieties have became a common subject of research in recent years.

Racker first postulated a possible metabolic pathway of deoxyribose by working with an <u>in vitro</u> enzyme system in 1952 Later, several workers (1-22) began using glucose-C^{14} tracer data to explain the possible metabolic pathway of deoxyribose biosynthesis. They found that the distribution of radioactivity in ribose and deoxyribose derived from glucose-C^{14} was very similar and suggested that deoxyribose is produced directly from ribose or that these two pentoses have the same precursor. They also found, in most cases, that the oxidative and nonoxidative pathways are both involved in pentose biosynthesis.

In previous studies, deoxyribose was degradated by <u>Escherichia coli</u> fermentation or by <u>in vitro</u> degradation with enzymes extracted from <u>E.coli</u> and rabbit muscle.

In 1962 Unrau and Canvin (53) developed a method to degrade deoxyribose by chemical procedures, but this method has not been compared to enzyme degradation procedures.

Almost all the studies in this field have been carried out with microorganisms and animal tissues. Similar information is lacking for plant materials.

The present study of deoxyribose biosynthesis was undertaken using plant material (wheat embryos) and the chemical degradation procedure of Unrau and Canvin (53).

LITERATURE REVIEW

Information on deoxyribose biosynthesis, especially in higher plants, is very sparse. Some enzymatic and radioisotopic studies in microorganisms and higher animals have shown that both glucose and ribose were precursors in deoxyribose biosynthesis. The complete metabolic pathway, however, is not clear.

Hypothesis for Ribose and Deoxyribose Biosynthesis

Two major pathways are known which can lead from hexose to ribose. The well known oxidative decarboxylation of glucose-6-phosphate, and the non-oxidative transaldolase and transketolase reactions which give rise to pentose phosphate.

The data, from microorganisms and animals, in most cases, showed that ribose was synthesized by both of these pathways. The relative contribution of each pathway was dependent on the species, culture media and other environmental factors.

Lanning and Cohen (1), Begatell *et al*. (2,3), and Bernstein and Sweet (4,5,) have shown in experiments with labeled hexose that ribose was synthesized by both pathways in *E.coli*. The oxidative pathway, however, was preferentially utilized. Similar results were obtained with *Candida utilized*. (6,7,8,9)

Szynkiewicz *et al*.(10) examined *E.coli* cultures grown under a variety of conditions. They found that the non-oxidative pathway was predominant when acetate was the principal source of carbon or when acetate grown cells were in the resting or stationary phase in a glucose medium. The oxidative pathway predominated, however, when the organism was in the logarithmic growth phase in a glucose medium. Wright *et al.* (11) studied the labeling pattern of deoxyribose isolated from synthesis of deoxyribose in bacteriophage infected *E.coli* by comparing labeling patterns in phage. Approximately 60% of the pentose appeared to have been synthesized by the oxidative pathway. The results were similar for deoxyribose from non-infected *E.coli*.

Fossit and Bernstein(12) studied *Pseudomonas saccharophila* and found that during aerobic growth on glucose, all the ribose is synthesized by a non-oxidative mechanism. Furthermore, gluconate-6-C^{14} produced pentose labeled almost equally in carbons one and five with very little label elsewhere. No definite explanation was proposed by the authors for this incorporation. Later on, Sable(13) suggested that it is possible that pentose is formed from glyceraldehyde-3-phosphate and hydroxypyruvate by the transketolase reaction.

A variation of the non-oxidative pathway is reported to exist in *Streptococcus faecalis*, which contains other enzymes of the pentose cycle but is almost completely devoid of transaldolase (14).

In animals; Kit *et al.*(15) incubated a suspension of cells derived from rat thymus, mouse spleen, rabbit appendix, lymphatic leucemia and Gardner lymphosarcoma with glucose-1-C^{14} and glucose-6-C^{14} and measured the incorporation of C^{14} into purine bound ribose of ribonucleic acid (RNA). The relative amounts incorporated from the different substrates suggested that in all these tissues a substantial portion of the pentose was synthesized by a non-oxidative route, which was first assumed to be the transketolase-transaldolase pathway.

In mouse liver, Shuster and Goldin (16,17) found that the non-oxidative pathway accounted for 90 to 95 % of ribose formation, whereas, only 5 to 10% was found to be produced by the oxidative pathway. Ghosh and Bernstein(18) found that one fourth of the ribose was formed by the oxidative pathway and three fourths by the non-oxidative pathway in rats. Hiatt (19) investigated the pathway in rats by the simultaneous administration of glucose-2-C^{14} and imidazole acetic acid. The ribose was produced from glucose-2-C^{14} and excreted in the urine as imidazole acetic acid riboside. By analysis of the isotope distribution in the ribose moiety of the excretion product it was demonstrated that 30 to 50% of the glucose was converted to ribose by the oxidative pathway while the remainder was produced non-oxidatively. This method enabled one to study ribose synthesis in an intact animal or human being. It was found that in humans approximately equal contributions of oxidative and non-oxidative pathways occurred (20).

In thiamine deficient rat, this proportion was strongly shifted in favor of the oxidative pathway (18,21). Thiamine pyrophosphate is known to be required for transketolase activity in rats (22).

Racker (23,24) postulated a deoxyribose phosphate aldolase which formed deoxyribose-5-phosphate from acetaldehyde and glyceraldehyde-3-phosphate. It has been shown that the synthesis of the deoxyribose moiety of deoxyribonucleic acid (DNA) can occur from two and three carbon precursors <u>in vitro</u> (1, 25, 26, 27, 28, 29). The above reaction, however, has always considered to be of little importance in the biosynthesis of deoxyribose <u>in vivo</u>, in view of the low affinity of the enzyme for acetaldehyde.(11,12,18,30). Deoxyribose aldolase probably catalyzes an important step in the breakdown of deoxyribose(31).

Many researchers have suggested that deoxyribose is converted directly from ribose while the latter is in the ribosidic linkage. Hammarsten <u>et al</u> (31) reported that cytidine, but not free cytosine, was incorporated into DNA. Rose and Schweigert (32,33), Roll <u>et al</u>. (34,35),Reichard (36) and Amos and Magasanik (37) showed that labeled pyrimidine ribonucleosides were incorporated into DNA with little or no change in relative labeling of base and pentose. The similar labeling of deoxyribose and ribose indicated that ribose was directly reduced to deoxyribose or at least, they had a common precursor (2,3,5,7, 10, 12,31) Itzhak and Writter (38,39) found the specific activities of the ribose and deoxyribose moieties bound to purine bases were greater than those bound to the corresponding pyrimidine bases.

Grossman and Hawkin (40) obtained cell free bacterial (*Salmonella typhimurium*) extracts which catalyzed the conversion of uridine and cytidine to deoxyuridine and deoxycytidine. Similar results with extracts from chick embryos were also observed by Reichard (41). The detailed enzyme mechanisms of the above reaction process has been reported by Moore and Hurlbert (42), Reichard (43,44) , and Abrams *et al*. (45). Recently, Bertani *et al*. (46) discovered that the actual conversion step of ribonucleotide to its corresponding deoxyribonucleotide occurred mostly at the diphosphate level. However, in animals,deoxyribose formation from the direct reduction of ribose is not certain. Horecker et al.(27) in comparable experiments on regenerating rat liver using glucose-1-C^{14} and glucose-2-C^{14}, found some differences between the labeling patterns in the two pentoses. Laland *et al*. (47) reported the ratio of incorporation of C^{14} from acetate-1-C^{14} and acetate-2-C^{14} was different in ribose and deoxyribose when rat liver slices were separately incubated with these two substrates. Ghosh and Bernstein (18) found that young adult rats had different labeling patterns in deoxyribose and ribose when they were fed glucose-C^{14}. Similar labeling patterns in these compounds were found, however, when new born rats were fed in the same way. It is possible that these differences might be a function of compartmentation of precursor pools or other environmental influences (27,48).

MATERIALS AND METHODS

Plant Material

Wheat embryos were isolated by a modification of the procedure of Johnston and Stern (49). Approximately 1000 g of wheat (*Triticum aestivum* var. *Pembina*) was frozen (-10 to -20° C) for one to two hours, the seed was ground for 25 to 30 seconds in a Lourdes tissue grinder. The mixture was screened through 12, 16 and 30 mesh sieves. The portion remaining on the 12 and 16 mesh sieves was returned at once to the grinder. The ground material was thoroughly shaken on the 30 mesh sieve. The portion which passed through the sieve contained wheat embryos and fragments of endosperm.

The embryos were separated from other fragments by stirring the mixture in 2 M sucrose solution. The suspension was allowed to settle and the embryos were skimmed off the surface. This step was repeated twice. These embryos were washed five times with sterilized water.

Radioactive Material

Glucose-U-C^{14} (125 mc/mMole), glucose-1-C^{14} (25.9 mc/mMole), glucose-2-C^{14} (28.4 mc/mMole), glucose-6-C^{14} (27.5 mc/mMole) were obtained from the Radiochemical Centre, Amersham, England. Each sample was taken up in 30 ml of water.

Incubation Conditions

Wheat embryos were grown at room temperature in Petri dishes containing radioactive glucose, for 48 hours. Each sample was fed approximately 3.5 μc. of labeled glucose.

Extraction of DNA

DNA was extracted by a modification of the procedure of Colter (50). After 48 hours incubation with radioactive glucose, embryos were removed and quickly washed with sterilized water several times. Then embryos were ground in a glass homogenizer with a small amount of phenol-buffer solution. The phenol-buffer solution contained 1.5 ml 10 % sodium deoxycholate in water, 28.5 ml 1 M sodium chloride in 0.02 M potassium-phosphate buffer, pH 7.4, and 30 ml water saturated phenol. The emulsion from the homogenizer was made up to 60 ml with phenol-buffer, then centrifuged for 10 minutes at 17,300 x g. The upper aqueous layer containing the DNA was carefully removed from the lower phenol phase. The DNA solution was subjected to two additional extractions with equal volumes of water saturated phenol. After low speed centrifugation at 4,340 x g, the aqueous layer was again removed. Traces of phenol in the aqueous layer were removed from the DNA by four or five brief ether extractions. The residual ether was eliminated by bubbling nitrogen through the solution. After adding an equal volume of 95% alcohol, DNA appeared as long fibers and was collected with a glass rod.

Degradation of DNA to Deoxyribonucleosides

The DNA was dissolved in 0.02 M $MgSO_4$ by adding 1 N

NH_4OH to a final pH of 7.6. A few crystals of deoxyribonucleoase (Nutritional Biochemicals Corporation) and a few drops of chloroform were added. The mixture was incubated at 37° C for 6 hours, NH_4OH was added to pH 9, glycine buffer (0.M glycine 50 ml and 8.8 ml NaOH make up to 200 ml) was added at a concentration of 5 % v/v. A few crystals of snake venom (Sigma Chemical Company) were added and the mixture was incubated for five hours. The mixture was centrifuged 4,340 x g and the supernatant containing deoxyribonucleosides was removed. (68).

Isolation of Deoxyribose

A few drops of bromine were added to the deoxyribonucleoside mixture, after 15 minutes the bromine was driven off by passing nitrogen through the solution. Then sodium hydroxide was added to pH 11, after one hour the solution was acidified with 1 N HCl to a final pH of 1.5. Hydrolysis was carried out at 100° C for 15 minutes. This procedure resulted in the removal of deoxyribose from purine and pyrimidine bases (51).

The hydrolysate was passed through Dowex 50 X 8 (H^+) and Duolite A-4 (OH^-) resin. The effulent contained the neutral sugars including deoxyribose. The procedure was tested on a sample of sperm DNA (Nutritional Biochemicals Corporation) using the diphenylamine method (52), as an estimate of deoxyribose content. A 50% recovery of deoxyribose was obtained by the above procedure, while only 33% recovery was obtained when treatment with bromine and NaOH was omitted from the procedures.

The solution was reduced in volume by vacuum evaporation, then applied to chromatogrphic paper in a narrow band 20 cm in length. The chromatogram was developed with ethyl acetate: acetate: water 8:2:2 (v/v) as the descending solvent for 16 to 20 hours. Two strips were cut from each side and sprayed with p-anisidine reagent (1.5 g p-anisidine and 5 g TCA dissolved in 125 ml distilled water). The deoxyribose in remaining non-sprayed central portions of the paper was eluted with distilled water and stored in the frozen state for further study.

<u>Degradation of Deoxyribose</u>

The degradation procedure of Unrau and Canvin(53)as modified as presented below: Sodium borohydride(30mg) was added to each sample of deoxyribose. The solution was maintained at pH 8-9 by addition of 0.5 N sulfuric acid until the rate of increase of pH became slow. The solution was kept at room temperature for two to three hours, then stored in a refrigerator overnight. The following day 0.5 N sulfuric acid was added to adjust to pH 5 . The solution was again stored in the refrigerator until complete destruction of excess sodium borohydride occurred. The colorimetric method for reducing group (54,55) was used to indicate the complete conversion of deoxyribose to the ribitol and the destruction of the sodium borohydride.

Oxidation of Deoxyribitol

The deoxyribitol was oxidized directly with sodium metaperiodate to formaldehyde, formic acid and β-hydroxypropionaldehyde. The completion of the reaction was determined on a duplicate sample by quantitatively determining the formaldehyde produced by the chromotropic acid method (56)

Sequential Degradation of the Mixture of Formic acid, Formaldehyde and β-hydroxypropionic acid

One milliliter of 25 % phosphoric acid and 3 g mercuric oxide were added to the sample which was then incubated at 75-85° C for six hours. Nitrogen was passed through the sample and the carbon dioxide produced from the formic acid was trapped in ethanolamine. (67)

The flask containing the reaction mixture was removed, the pH adjusted to 7, four or five drops of bromine were added and the flask was stoppered tightly. The sample was shaken for one hour, then 25% phosphoric acid and additional mercuric oxide were added and the evolved carbon dioxide from formaldehyde was trapped as previously.

The solution was then made alkaline (pH 11) and 5 ml of 5% potassium permanganate was added. After 45 minutes the solution was acidified and the carbon dioxide was trapped. This carbon dioxide was derived from the carboxyl carbon of β-hydroxypropionic acid. The oxidation mixture containing the two carbon fragment was left overnight at 40° C to facilitate oxidation of the initial product, ethanol, to acetic acid. The acetic acid was extracted with ether and the ether fraction evaporated almost to dryness. The residue was made alkaline by addition of a few drops of 0.5 N NaOH and the solution evaporated to dryness followed by overnight drying at 100° C. Sodium acetate was degraded by the Schmidt and Phares method (57). Concentrated sulfuric acid (0.2 ml) was added to the sample, sodium azide (30 mg) was then added and the flask was connected to a gas train containing 5% potassium permanganate in 5 % sulfuric acid to remove sulfur dioxide. The flask was heated on a water bath at 75-80° C for one hour. The system was flushed with nitrogen for 15 minutes and the carbon dioxide was collected in ethanolmine.

A solution of 5 % potassium permanganate (5 ml) was added to the residue and the pH raised to 11 with 40 % NaOH. The mixture was heated for 15 minutes on a boiling water bath. The system was flushed with nitrogen and the reaction mixture acidified with sulfuric acid to release carbon dioxide which was again trapped in ethanolamine.

Studies of the mechanism of alkaline permanganate decarboxylation of β-hydroxypropionic acid were carried out and the information published (58). A reprint of the paper can be found in the appendix.

Assay of Radioactivity

Carbon dioxide from the degradation procedures was assayed by flushing the reaction vessel with carbon dioxide free nitrogen and passing the exit gases through a solution of ethanolamine: methylcellosolve 1:2 (5 ml).

A one ml aliquot of the ethanolamine which contained the radioactive carbon dioxide was transferred to a glass counting vial and 15 ml organic scintillator solution was added. The scintillator solution contained 6 g diphenyloxazole (PPO) in toluene:methylcellosolve 2:1 (v/v). Radioactivity was determined with a Nuclear Chicago Model 724 liquid scintillation spectrometer. Background corrections were made automatically and the observed count rate was corrected to an absolute value (disintegrations per minute) by the channel ratio method. Counting error was less than 5%.

Experimental Degradation of Deoxyglucose

Information on the recovery of carbon one and two of deoxyribose is available (53) on the basis of non-labeled material. These values, however, tend to be variable because of carbon dioxide contamination in the alkaline reagents utilized. More accurate determinations, however, could be obtained if a source of uniformly labeled deoxyribose were available.

The recovery of carbons three, four, and five of deoxyribose, on the otherhand, can be more readily estimated using

TABLE I

EXPERIMENTAL DEGRADATION OF DEOXYGLUCOSE

Experiemnt No.	Experimental and Calculated Yields, Respectively from Deoxyglucose Carbon		
	3	4+5	6
1	8,7 (9.1)	13.5 (18.2)	13.2 (9.1)
2	7.8 (7.6)	9.6 (15.1)	19.4 (7.6)
3	8.6 (8.0)	12.1 (16.0)	13.0 (8.0)
4	5.3 (6.5)	10.6 (12.9)	7.8 (6.5)
Totals	30.4 (31.2)	45.8 (62.2)	53.4 (31.2)
% Recovery	98	74	170
Correction Factor	1.00	1.43	

non-labeled deoxyribose (Table I).

The incomplete recovery of carbons four and five of deoxyglucose (Table I) resulted in a high experimental value for carbon six which is determined in the subsequent step. On the basis of these data corrections are made for carbons four and five of deoxyribose in Tables II-V . Values for carbon four of deoxyribose were multiplied by 1.43 (Carbon four plus carbon five of deoxyglucose are equivalent to carbon four of deoxyribose). Experimental values for carbon five of deoxyribose were decreased by the amount added as correction factor for carbon four.

RESULTS AND DISCUSSION

Theoretical Considerations

In order to evaluate the experimental data which will be presented, let us first consider in more detail previous studies of deoxyribose biosynthesis.

Previous studies with microorganisms and animals showed that there are two principal pathways for the biosynthesis of pentose from hexose. The most direct route for the synthesis of pentose from hexose is the oxidative pathway which involves the decarboxylation of 6-phosphogluconate to ribulose-5-phosphate. The stoichiometry of the overall reaction is as follows

$$3 \text{ hexose} + O_2 \longrightarrow 3 \text{ pentose} + 3\ CO_2.$$

By this pathway, carbon two through carbon six respectively of glucose furnishes carbon one through carbon five respectively of ribose, while carbon one of glucose is lost as carbon dioxide. If recycling occurred, redistribution of first three carbon atoms from glucose would take place during the second, third and subsequent cycles. The redistribution pattern is summarized in Scheme I.

The second route is the non-oxidative synthesis of pentose from hexose which involves the reactions catalyzed by transaldolase and transketolase. The reactions are summarized as follows.

1. Fructose-6-phosphate + glyceraldehyde-3-phosphate $\xrightarrow{\text{transketolase}}$ pentose-5-phosphate + erythrose-4-phosphate.
2. Fructose-6-phosphate + erythrose-4-phosphate $\xrightarrow{\text{transaldolase}}$

glyceraldehyde-3-phosphate + sedoheptulose-7-phosphate.

3. Glyceraldehyde-3-phosphate + sedoheptulose-7-phosphate

$\longrightarrow$ 2 pentose-5-phosphate.

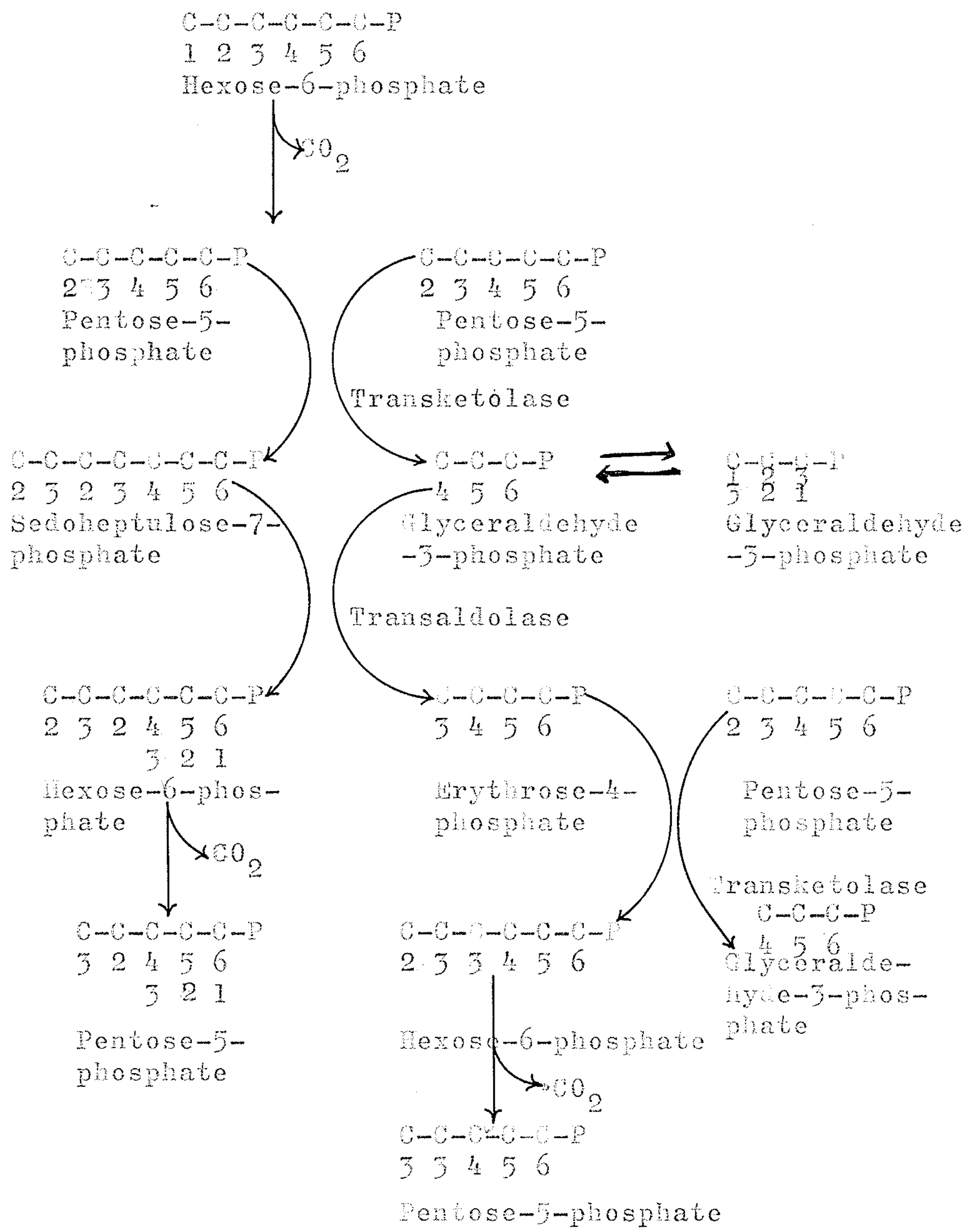
Sum: 2 fructose-6-phosphate + glyceraldehyde-3-phosphate

$\longrightarrow$ 3 pentose-5-phosphate.

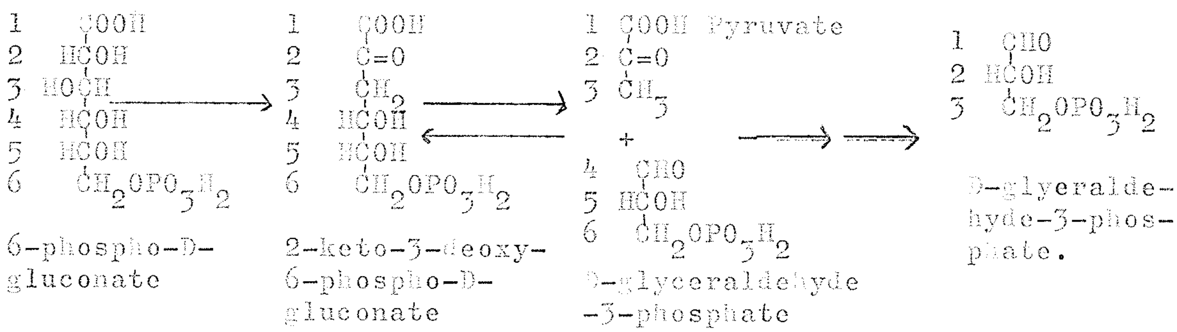
The above reactions require stoichiometric quantities of glyceraldehyde-3-phosphate, which could arise in different manners. The metabolic sequence giving rise to glyceraldehyde will determine, to a great extent, the possible disposition of carbon from glucose to deoxyribose. If glyceraldehyde phosphate is produced by the pentose phosphate cycle (see Scheme I), then the three carbons of this triose are derived solely from carbon four, five and six of glucose. On the other hand, if glucose is also metabolized by the Embden-Meyerhof-Parnas (EMP) pathway, glyceraldehyde-3-phosphate could arise from carbon one, two,three or four, five and six of glucose. The following carbon atoms of glucose become equivalent in the triose as a result of the triose phosphate isomerase reaction: one and six, two and five, and three and four.

If the Entner-Doudoroff pathway (see Scheme II) takes part in the metabolism, then glyceraldehyde-3-phosphate would be derived from carbon one,two and three of glucose, and carbon one of glyceraldehyde-3-phosphate would correspond to carbon one of glucose.

SCHEME I OXIDATIVE PATHWAY



SCHEME II ENTNER-DOUDOROFF PATHWAY



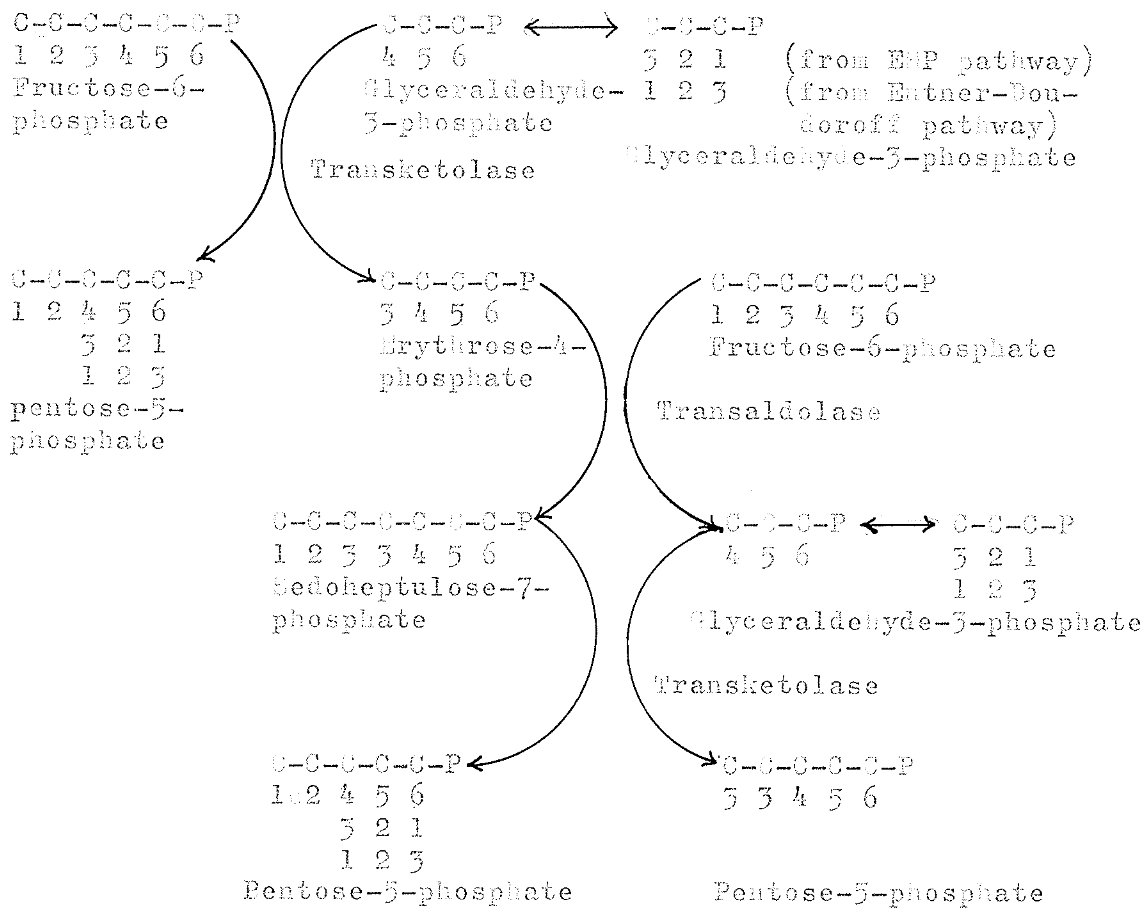
If glyceralde-3-phosphate originates from the EMP or Entner-Doudoroff pathway and is utilized in the production of pentose via transaldolase and transketolase reactions, then the distribution of carbon atoms from glucose in the pentose molecule would be as presented in Scheme III.

An alternate pathway is the condensation of acetaldehyde and triose phosphate. In the biosynthesis of triose phosphate, the following carbons of glucose become equivalent, three and four, two and five, and one and six. These give rise to carbons one, two and three respectively of triose phosphate . Carbon one of acetaldehyde may arise from carbon one or five of glucose and carbon two from two or six of glucose. The distribution of carbon atoms in the pentose molecule from glucose is presented in Scheme IV.

The low affinity of deoxyribose phosphate aldolase for acetaldehyde suggests that this enzyme may be important in the degradation but not in the synthesis of deoxyribose.

Another pathway is from glucose via glucuronic acid and xylulose with loss of carbon six of glucose. This cycle was

SCHEME III NON-OXIDATIVE PATHWAY



SCHEME IV THREE TWO CONDENSATION

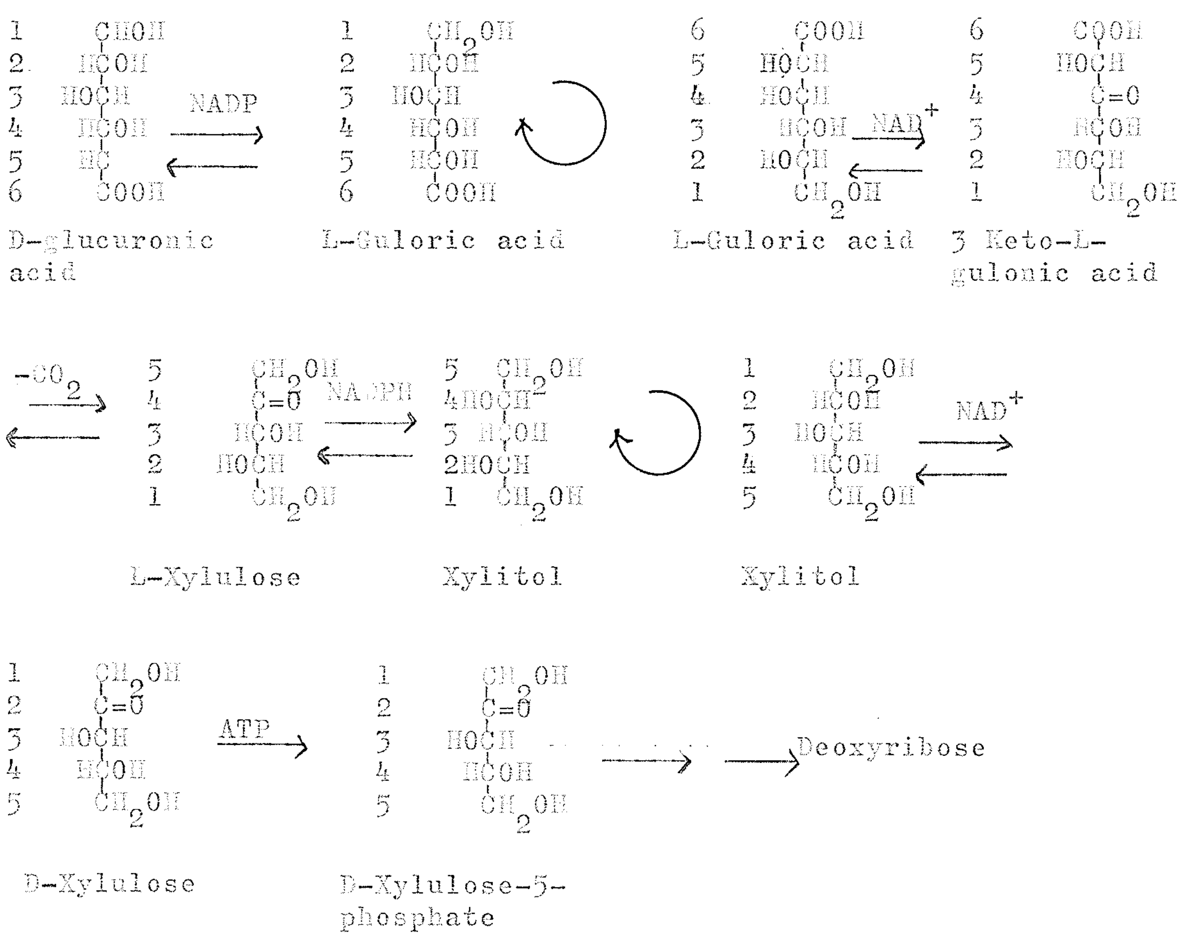
5 1 CHO
6 2 CH_3
Acetaldehyde
+
3 4 CHO
2 5 CHOH
1 6 $CH_2OPO_3H_2$
Glyceraldehyde-3-phosphate

$\xrightarrow{\text{deoxyribose phosphate aldolase}}$
$\longleftarrow$

1 CHO
2 CH_2
3 CHOH
4 CHOH
5 $CH_2OPO_3H_2$
Deoxyribose-5-phosphate

first suggested in 1956 (59) and some experiments (60,61,62 63,64) support the theory that carbon six of glucose is converted into carbon dioxide. But these experiments do not suggest that this glucuronate xylulose cycle has any significant quantitative role in carbohydrate metabolism. The reactions of this cycle and the distribution of carbon atoms is summarized in Scheme V.

SCHEME V GLUCURONATE -XYLULOSE CYCLE



Experimental

Glucose-1-C^{14}, glucose-2-C^{14}, glucose-6-C^{14}, and glucose-U-C^{14} are efficient precursors of deoxyribose. C^{14} distribution on each carbon of deoxyribose derived from these precursors was used in attempts to determine the biosynthetic pathway of deoxyribose.

Wheat embryos were incubated with C^{14} labeled glucose for 48 hours, deoxyribose was isolated and degraded. The results are presented in Tables II - VI.

Non-radioactive deoxyribose (3 mg) was added to the sample before degradation. The specific activities reported are the values after dilution. All data in these Tables are presented as disintegrations per minutes (dpm). The multiple range test (66) was used to analyze the data.

Glucose-U-C^{14} feeding experiments

Eight feeding experiments were carried out and the distribution of C^{14} activity in deoxyribose derived from glucose-U-C^{14} was determined. (Table II)

In these experiments the activity of carbon one and carbon two of deoxyribose was statistically lower than the activity of carbon four and five. From a theoretical point of view, if the five carbons of deoxyribose all originate from glucose-U-C^{14}, the activity of each carbon of deoxyribose

TABLE II

C^{14} DISTRIBUTION IN DEOXYRIBOSE FROM GLUCOSE-U-C^{14}

Experiment No.	Radioactivity of Deoxyribose (dpm)					Specific Activity of Deoxyribose dpm/mMole***
	C_1	C_2	C_3	C_4	C_5	
1	52	50	56	75	36	19880
2	40	44	46	70	126	"
3	40	44	46	75	68	"
4	87	82	41	129	82	"
5	58	68	43	44	76	"
6	33	30	78	57	34	103869
7	55	30	58	61	46	"
8	32	41	68	97	108	"
Mean	46^a	48^a	54^{ab}	76^b	72^b	
%Activity	15.7	16.6	18.6	25	24	

* Sum of values in each column divided by the grand total.

** Means having an identical superscript are not significantly different at the 5% level of significance.

*** The specific activities reported are the values after dilution with 3 mg non-radioactive deoxyribose.

should be the same. The lower values of these two carbons, however, may be explained in the following manner. Firstly, carbon one and carbon two may originate from pools of non-radioactive precursors rather than from glucose-U-C^{14}. Secondly a portion of the activity of carbon one and carbon two of deoxyribose may be lost during the degradation procedure (see page 12-16).

If one assumes that glucose-U-C^{14} feeding should result in uniform labeling of deoxyribose, then one might correct the label distribution pattern of deoxyribose obtained when glucose-1, 2 and 6 C^{14} are fed. This procedure, however, had little effect on the difference observed in the data as presented Table V. Corrected data of Table V shows the relatively unimportant changes in C^{14} distribution in deoxyribose as a result of such correction(Table VI).

Glucose-6-C^{14} feeding experiments

Six feeding experiments were carried out and the distribution of C^{14} activity in deoxyribose derived from glucose-6-C^{14} is shown in Table III.

Of the four possible metabolic pathways of deoxyribose biosynthesis previously discussed, (Scheme IV) the condensation of acetaldehyde and glyceraldehyde-3-phosphate by the enzyme deoxyribose phosphate aldolase has received little support.

The enzyme affinity for acetaldehyde is too low to form any significant amount of product in vivo (11,12,18,30,31). The glucuronic acid xylulose cycle(Scheme V) appears to be relatively unimportant for the biosynthesis of deoxyribose and ribose, at least in animals(65). Thus in this study only the oxidative pathway (Scheme I) and non-odidative pathway (Scheme III) are considered.

Almost 50 % of the radioactivity derived from glucose-6-C^{14} was found in carbon five of deoxyribose (Table III). If deoxyribose was synthesized by the oxidative pathway, carbon two through carbon six respectively of glucose would provide carbon one through carbon five respectively of deoxyribose, whereas carbon one of glucose would be lost as carbon dioxide. The C^{14} activity of glucose-6-C^{14} would therefore occur on carbon five of deoxyribose. If the non-oxidative pathway is operative in deoxyribose biosynthesis, then one would expect radioactivity from glucose-6-C^{14} to be contributed to carbon five of deoxyribose (Scheme III).

In Table III, the radioactivity of carbon five is higher than that of the other four carbons of deoxyribose. This is in agreement with biosynthesis via Scheme I or III. The radioactivies of carbons three and four, however, are higher than those of one and two. If Scheme I and Scheme III are combined then after recycling radioactivity could occur in position three and four of deoxyribose derived from glucose-6-C^{14}.

TABLE III

C^{14} DISTRIBUTION IN DEOXYRIBOSE FROM GLUCOSE-6-C^{14}

Experiment No.	Radioactivity of Deoxyribose (dpm)					Specific Activity of Deoxyribose dpm/mMole
	C_1	C_2	C_3	C_4	C_5	
1	10.0	18.5	117.3	76.4	348	21528
2	24.5	25.0	136.0	184.3	248.0	"
3	20.5	45.0	40.1	100.0	141.0	16458
4	17.0	31.5	60.0	72.0	158.0	"
5	10.0	22.5	49.0	34.0	144.3	"
6	18.0	27.5	172.0	117.9	313.1	
Mean	16.7[a]	28.3[a]	95.8[b]	97.4[b]	225.4[c]	
% activity	5.6	5.9	20.3	20.4	49	

Glucose-1-C^{14} feeding experiments

Seven feeding experiments were carried out and the C^{14} distribution of deoxyribose derived from glucose-1-C^{14} determined (Table IV).

In the data of Table IV, approximately 42% of the radioactivity in deoxyribose is on carbon five. Carbon three and carbon four also showed some radioactivity. Radioactivity in these two carbons may have resulted from a significant amount of recycling during the feeding period.

If the oxidative pathway was involved in deoxyribose biosynthesis, carbon one of glucose would be converted into carbon dioxide and no significant amount of label in deoxyribose would be found. The data show relatively high total specific activities for deoxyribose, thus the oxidative pathway appeared to be relatively unimportant for deoxyribose biosynthesis. However, some radioactivity could appear in deoxyribose, if glyceraldehyde-3-phosphate from the EMP cycle entered into the oxidative pathway.

If the non-oxidative pathway was active in deoxyribose biosynthesis, then radioactivity would be expected on carbon two of deoxyribose. If on the otherhand, the non-oxidative pathway operated in conjunction with the EMP pathway, then the label would be expected on carbon two and five. Data in Table IV indicated high radioactivity on carbon five but not on carbon two of deoxyribose.

TABLE IV

C^{14} DISTRIBUTION IN DEOXYRIBOSE FROM GLUCOSE-1-C^{14}

Experiment No.	Radioactivity of Deoxyribose (dpm)					Specific Activity of Deoxyribose dpm/mMole
	C_1	C_2	C_3	C_4	C_5	
1	16.0	35.5	102.0	149.0	306.0	18061
2	38.0	76.0	55.5	118.0	104.5	9135
3	12.0	30.0	75.0	117.0	103.5	"
4	14.0	44.0	71.5	85.7	112.0	"
5	13.0	28.5	100.0	64.5	128.0	"
6	37.4	27.0	61.4	98.2	243.2	18061
7	14.0	44.0	62.5	52.5	152.0	9135
Mean	20.6^{a}	40.7^{ab}	74.4^{bc}	89.4^{c}	164.2^{d}	
% Activity	5.4	14.	19.6	23.0	41.8	

Glucose-2-C^{14} feeding experiments

Eight feeding experiments were carried out and C^{14} activity distribution of deoxyribose derived from glucose-2-C^{14} is shown in Table V. The data in Table V shown 39 % of total activity on carbon four and 30 % of total activity on carbon five of deoxyribose.

If the non-oxidative and EMP pathways are involved in the biosynthesis of deoxyribose, carbon one and carbon four will have high radioactivity. The high radioactivity of carbon four (Table V) is in agreement with this hypothesis, while the low radioactivity of carbon one could be due to arguments preciously presented. Labeling of carbon five, however, cannot be explained by this system, irregardless of the degree of recycling. The oxidative pathway must be invoked to explain labeling of carbon five of deoxyribose from glucose-2-C^{14}.

C^{14} distribution in deoxyribose from glucose-2-C^{14} (Table V) corrected on the basis of Table II is shown in Table VI. Corrected data of Table V, however, shows the relatively unimportant changes in C^{14} distribution in deoxyribose as a result of such correction (Table VI).

TABLE V

C^{14} DISTRIBUTION IN DEOXYRIBOSE FROM GLUCOSE -2-C^{14}

Experiment No.	Radioactivity of Deoxyribose (dpm)					Specific Activity of Deoxyribose dpm/mMole
	C_1	C_2	C_3	C_4	C_5	
1	31.0	20.5	38.5	50.7	57.3	3924
2	19.0	9.5	43.0	51.4	63.1	"
3	13.0	11.6	32.5	91.8	74.5	6450
4	20.0	27.5	50.0	133.6	90.9	11571
5	29.5	13.3	56.3	131.4	110.1	"
6	12.5	20.0	10.0	77.9	21.7	3924
7	25.5	29.5	22.5	33.6	19.4	3257.6
8	30.0	13.0	23.2	180.7	122.8	43884
Mean	22.6[a]	18.1[a]	34.5[a]	93.9[b]	69.9[b]	
% Activity	9.5	7.5	14.1	39.0	30.0	

TABLE VI

C^{14} DISTRIBUTION IN DEOXYRIBOSE FROM GLUCOSE-2-C^{14} CORRECTED ON THE BASIS OF TABLE II.

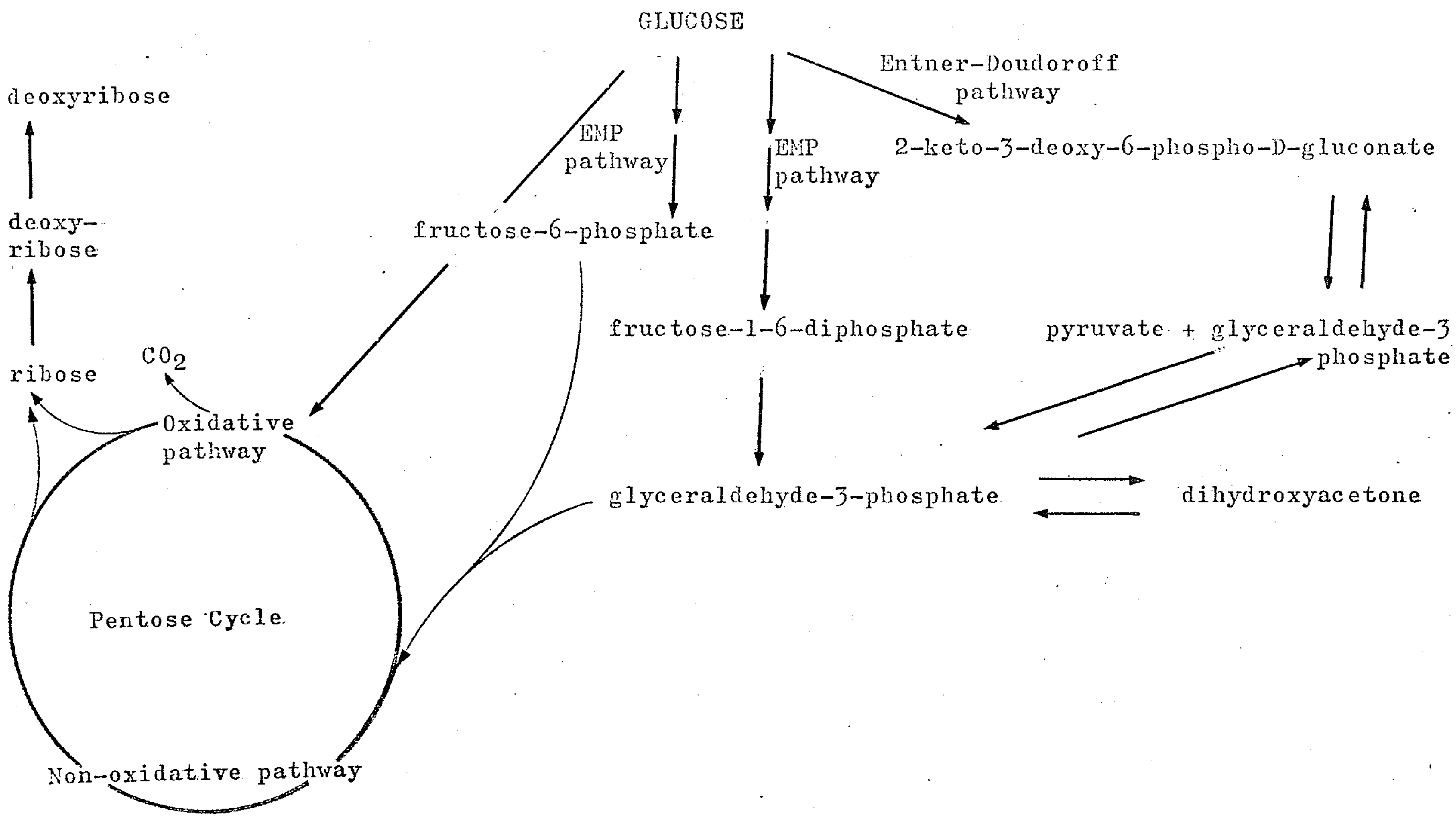
Experiment NO.	Radioactivity of Deoxyribose (dpm)				
	C_1	C_2	C_3	C_4	C_5
1	60.8	38.8	64.9	57.3	72.5
2	37.3	18.0	72.5	58.1	78.5
3	25.5	21.9	54.8	105.7	102
4	39.2	52;1	84.3	150.9	151
5	57.8	25.1	94.8	148.5	149
6	24.5	37.9	16.8	87.9	45
7	50.0	35.9	37.9	37.9	29.5
8	58.[illegible]	24.6	59.0	204.1	177.0
Mean	44.24[a]	34.0[a]	58.1[a]	106.1[b]	97.1[b]

Possible Pathway of Deoxyribose Biosynthesis

Previous research has, in general, indicated that both ribose and deoxyribose are synthesized via the same pathways or from common precursors (2,3,4,7, 10, 12, 31-36). In most cases, both ribose and deoxyribose bear a similar labeling pattern when derived from glucose-C^{14}. A notable exception to this was found by Horecker et. al. (27) and Laland et. al. (47) in their work with animal tissue. This biosynthesis of ribose appeared to be via a combination of the oxidative and non-oxidative pathways (1-20) with a variation in the degree of participation dependent on the organism studied.

The data presented offer no definite conclusions as to whether or not deoxyribose arises from a reduction of carbon two of ribose, since no studies of the labeling pattern of ribose were carried out. These results, nevertheless, bear a close similarity to the deoxyribose labeling pattern presented by previous workers(27, 47), and one might assume same similarity in pathway between animals and higher plants.

The C^{14} distribution pattern of deoxyribose presented in Tables II-V, can be at least partially explained if one assumes a combination of the oxidative and non-oxidative pathway gives rise to deoxyribose. In such a sequence of metabolic events triose phosphate becomes a key compound, since it may be derived both from the EMP pathway and via the Entner-Dodoroff pathway



Scheme 6. Possible pathway of 2-deoxyribose biosynthesis

(scheme II). This combined system is presented in Scheme VI.

In general, the amounts of radioactivity found on carbons one and two of deoxyribose are low, even if these values are corrected on the basis of C^{14} distribution in deoxyribose derived from glucose-U-C^{14}. This would lead one to suggest that other pathways may be involved in deoxyribose biosynthesis in higher plants.

SUMMARY

A study of the biosynthesis of deoxyribose was carried out by supplying glucose-U-C^{14}, glucose-1-C^{14}, glucose-2-C^{14}, and glucose- 6-C^{14} to embryos from wheat (*Triticum aestivum*). After 48 hours incubation with C^{14} label substrates, DNA was isolated by the phenol method. Deoxyribose was released from DNA by a combined enzyme and acid hydrolysis procedure.

Degradation of deoxyribose was carried out using a modified version of the procedure of Unrau and Canvin. C^{14} activity was determined by liquid scintillation spectrometry The following results were obtained.

1. When glucose-6-C^{14} and glucose-1-C^{14} were supplied as substrates, the highest amount of radioactivity was found on carbon five of deoxyribose.
2. Glucose-2-C^{14} feeding resulted in the highest amount of radioactivity on carbon four of deoxyribose, with considerable radioactivity also on carbon five.
3. The non-oxidative pathway may play a dominant role in the biosynthesis of deoxyribose. The oxidative pathway, however, also appears to be important in deoxyribose biosynthesis. Glyceraldehyde -3-phosphate produced in glycolysis may be a key intermediate for both pathways.
4. Possible pathways of deoxyribose biosynthesis in wheat embryos are presented.

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ALKALINE PERMANGANATE DECARBOXYLATION OF β-AMINO- AND β-HYDROXY-PROPIONIC ACID

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ALKALINE PERMANGANATE DECARBOXYLATION OF β-AMINO- AND β-HYDROXY-PROPIONIC ACID

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In a previous communication (1), it was reported that the alkaline permanganate oxidation of β-hydroxypropionic acid gave a quantitative yield of carbon dioxide upon acidification. Furthermore, the remaining two-carbon fragment was acetate, and the correct analytical values were obtained in a Schmidt degradation (2); also, 2 equivalents of carbon dioxide was obtained by oxidation with persulfate. The possible oxidation of β-hydroxypropionic acid to malonic acid had, however, not been considered.[3] If malonate was indeed formed, then the degradative procedure developed for the sequential degradation of 2-deoxy-D-ribose (1) would obviously be of much reduced value in biosynthesis experiments involving ^{14}C-labelled metabolic intermediates. It was necessary, therefore, to establish the reaction sequence involved in the alkaline permanganate decarboxylation of β-hydroxypropionic acid. A simple approach to a solution of this problem entailed the decarboxylation of compounds appropriately labelled with ^{14}C.

β-Alanine-1-^{14}C was commercially available and was subsequently used in the preparation of β-hydroxypropionic acid-1-^{14}C. Decarboxylation of unlabelled β-alanine gave a fully quantitative yield of carbon dioxide (Table I). Since it was possible that this compound might also be oxidized to malonic acid before decarboxylation, it was necessary to degrade the appropriate ^{14}C-labelled compound.

Decarboxylation of carboxyl-labelled β-alanine (Table II) gave 85 to 90% $^{14}CO_2$ in four different experiments, thus indicating that a concerted elimination of the carboxyl carbon occurred by a mechanism probably involving a β-elimination in the semialdehyde intermediate.

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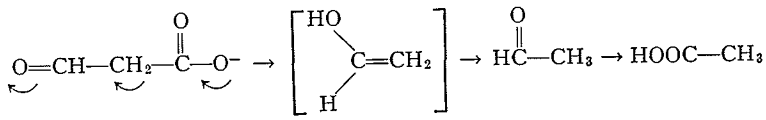
Decarboxylation of β-hydroxypropionic acid-1-^{14}C gave similar results, in that, in four experiments, the ^{14}C-labelled carboxyl carbon was essentially eliminated quantitatively. This observation thus shows that the alkaline permanganate decarboxylation likely proceeds via a β-elimination mechanism at the intermediate semialdehyde stage, as illustrated below. The first reaction product, apart from carbon dioxide, would be acetaldehyde, which is then further oxidized to acetate. In previous experiments (1) involving



unlabelled starting compound, a Schmidt degradation of the residue from the permanganate decarboxylation gave the expected analytical values. When these experimental results are considered, the methods and reactions described earlier (1) for the sequential degradation of 2-deoxy-D-ribose are further established and verified.

EXPERIMENTAL

Synthesis of β-Hydroxypropionic Acid-1-^{14}C from β-Alanine-1-^{14}C

A concentrated aqueous solution of sodium nitrite (2 moles of $NaNO_2$ per mole of amino acid) was added slowly to an ice-chilled solution of β-alanine (117 mg unlabelled β-alanine and 6×10^5 d.p.m. β-alanine-1-^{14}C) in 50% aqueous acetic acid under a nitrogen atmosphere. The chilled mixture was stirred for 40–48 h, the solution concentrated under reduced pressure to a small volume, and the concentrate applied as a narrow band on sheets of Whatman No. 1 paper. Standard β-hydroxypropionic acid was used as a marginal marker. Chromatography was carried out with ethyl acetate – acetic acid – water (8:2:2 v/v/v) as the eluent and with a developing time of 16 h. The paper was dried in a stream of warm air until free of acetic acid. Two marginal guide strips were cut and sprayed with bromocresol green reagent. The β-hydroxypropionic acid in the remaining unsprayed central portion of the paper was eluted with water. The percentage yield of β-hydroxypropionic acid synthesized from β-alanine was found to be 34.4. This was determined by comparing the disintegrations per minute of the mixture before chromatography (8 825 d.p.m.) and the disintegrations per minute of the β-hydroxypropionic acid eluted from the paper chromatograms (3 040 d.p.m.).

Decarboxylation with Potassium Permanganate

Unlabelled β-Alanine

β-Alanine (10.25 mg) was dissolved in distilled water (5 ml) in a 50 ml distillation flask. The solution was made neutral, and then 3 ml alkaline permanganate solution (consisting of 0.1 *M* NaOH, 0.2 *M* $KMnO_4$, and 0.02 *M* $MnSO_4$) was added. The flask was fitted to an aeration train and the contents held at room temperature for about 45 min. Before the solution was acidified, the train was flushed with nitrogen for 10 to 15 min. The solution was then acidified (2 *N* H_2SO_4, 2 ml), and the CO_2 that was evolved was swept into a $Ba(OH)_2$ trap. The precipitate that formed ($BaCO_3$) was collected on tared porcelain planchets, dried, and weighed. The results are given in Table I.

TABLE I

Permanganate decarboxylation of β-alanine

Weight of β-alanine (mg)	Weight of $BaCO_3$ expected (mg)	Weight of $BaCO_3$ obtained (mg)
21.4	44	43.6
25.1	52	51.8
19.6	39	34.2

β-Alanine-1-^{14}C

The procedure used was the same as described above, except that the CO_2 was swept into an ethanolamine trap (ethanolamine – methyl cellosolve (1:2 v/v)). The radioactivity was determined on a liquid scintillation counter. The results are given in Table II.

β-Hydroxypropionic Acid-1-^{14}C

The procedures employed were the same as described for the decarboxylation of β-alanine-1-^{14}C. The results are given in Table III.

TABLE II

Percentage recovery of $^{14}CO_2$ from permanganate decarboxylation of β-alanine-1-^{14}C

Disintegrations per minute expected	Disintegrations per minute obtained	$^{14}CO_2$ recovered (%)
4 974	4 266	87
4 974	4 225	85
4 974	4 450	89.7
4 974	4 320	87.5

TABLE III

Percentage recovery of $^{14}CO_2$ from permanganate decarboxylation of β-hydroxypropionic acid-1-^{14}C

Disintegrations per minute expected	Disintegrations per minute obtained	$^{14}CO_2$ recovered (%)
6 080	5 050	83
6 080	6 324	107
6 080	6 650	110
6 080	6 240	102

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