Jeanloz, R. W., Sharon, N., and Flowers, H. M. (1963), *Biochem. Biophys. Res. Commun. 13*, 20.
Jeanloz, R. W., Walker, E., and Sinaÿ, P. (1968), *Carbohydrate Res. 6*, 184.
Osawa, T., and Jeanloz, R. W. (1965), *J. Org. Chem. 30*, 448.
Reissig, J. L., Strominger, J. L., and Leloir, L. F. (1955), *J. Biol. Chem. 217*, 959.
Salton, M. R. J., and Ghuysen, J.-M. (1959), *Biochim. Biophys. Acta 36*, 552.
Salton, M. R. J., and Ghuysen, J.-M. (1960), *Biochim. Biophys. Acta 45*, 355.
Sharon, N., Osawa, T., Flowers, H. M., and Jeanloz, R. W. (1966), *J. Biol. Chem. 241*, 223.
Sharon, N., and Seifter, S. (1964), *J. Biol. Chem. 239*, PC 2398.
Sinaÿ, P., and Jeanloz, R. W. (1969), *Carbohydrate Res. 10*, 189.
Tipper, D. J., Ghuysen, J.-M., and Strominger, J. L. (1965), *Biochemistry 4*, 468.

Pentose Synthesis in *Escherichia coli**

Richard Caprioli† and David Rittenberg

ABSTRACT: *Escherichia coli* was grown on [1-^{18}O]-, [2-^{18}O]-, and [6-^{18}O]glucose and [2-^{18}O]fructose as the sole carbon sources. Growth was terminated in midexponential phase and the nucleic acids were isolated and degraded to the nucleoside level. The distribution of ^{18}O in the nucleosides was determined in a mass spectrometer by observing mass shifts of fragment ions in the mass spectrum of the nucleosides. A fragment ion containing ^{18}O exhibits a peak in the mass spectrum 2.0043 mass units higher than the normal ion containing ^{18}O. The relative intensities of the peaks can be related to the per cent ^{18}O in the fragment. When [1-^{18}O]- and [6-^{18}O]-glucose were used as carbon sources, 35 and 64%, respectively, of the original label of the hexose appeared in the 5′-oxygen atom of the nucleosides. No other oxygen atoms were labeled. When [2-^{18}O]glucose and [2-^{18}O]fructose were used as the substrates, the ribosides were similarly labeled with approximately 14% of the original label of the hexose in the 2′ position and 22% in the 4′ position (the deoxyribosides contained ^{18}O only in the 4′-oxygen atom). These results show that both the oxidative and nonoxidative pathways operate simultaneously to produce pentose phosphate. The major portion (about 70%) of the pentose in the nucleic acids was synthesized *via* the nonoxidative pathway and the remainder *via* the oxidative pathway. The above experiments also provide evidence which suggests that the enzyme aldolase in *E. coli*, in contrast to that of mammalian muscle, cleaves fructose 1,6-diphosphate without the obligatory loss of the C-2 oxygen atom.

D-Glucose is commonly utilized by living cells as a source of energy and intermediates necessary for the biosynthesis of vital cellular components. For the conversion of glucose into ribose, two major biosynthetic pathways coexist in most living cells; the oxidative pathway and the nonoxidative pathway.

When glucose is metabolized *via* the oxidative pathway to pentose, the reactions are accompanied by the formation of 2 moles of TPNH/mole of hexose utilized. The result is the conversion of 1 mole of hexose into 1 mole of pentose and 1 mole of CO_2.

When glucose is metabolized by the nonoxidative pathway, the reactions result in the conversion of 2.5 molecules of hexose phosphate into 3 molecules of pentose phosphate.

In addition to the oxidative and nonoxidative pathways, there are several other pathways by which pentose may be produced. The Entner–Doudoroff pathway produces glyceraldehyde 3-phosphate and pyruvic acid from hexose through the intermediate 2-keto-3-deoxy-6-phosphogluconate (Entner and Doudoroff, 1952). Pentose could be formed from this triose through the action of transketolase. However, it has recently been shown (Fraenkel and Levisohn, 1967; Zablotny and Fraenkel, 1967) that *Escherichia coli* mutants lacking gluconate 6-phosphate dehydrase and phosphoglucose isomerase do not utilize the Entner–Doudoroff pathway when grown on glucose. A second minor pathway for glucose metabolism, demonstrated in animal tissue, is the glucuronic acid pathway. In this pathway, glucose may be converted into ribose through the formation of 3-keto-L-gulonic acid, an intermediate in ascorbic acid biosynthesis (Hassan and Lehninger, 1956). However, Hiatt and Lareau (1958) have obtained evidence to show that it does not play a role in ribose biosynthesis.

The concurrent operation of the oxidative and nonoxidative

* From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York. *Received April 15, 1969.* This work was supported in part by grants from the National Institutes of Health (No. AM-08625) and the Atomic Energy Commission (No. AT(30-1)1803).

† Predoctoral fellow of the National Institutes of Health (No. Fl-GM-37691-10A1). This work has been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

pathways provides a means for the synthesis and reutilization of ribose 5-phosphate, depending upon the needs of the cell. The oxidative pathway generates 2 moles of TPNH/mole of glucose metabolized. This reduced form of the coenzyme is necessary for many reductive biosynthetic reactions, such as in the formation of deoxyribonucleotides, fatty acids, and certain amino acids as well as other compounds. The formation of TPNH may be one of the major functions of the oxidative pathway.

During the past 15 years, much work has been done with ^{14}C-labeled glucoses in an attempt to determine the relative contributions of the two pathways for glucose metabolism. In these experiments, cells were grown on glucose specifically labeled with ^{14}C in various positions. The ribose and deoxyribose portion of the nucleic acids was isolated and degraded to determine the labeling pattern. The interpretation of these results led to estimations of between 10 and 80% for the relative participation of the oxidative pathway for ribose synthesis in *E. coli*. This wide range was due, in part, to the fact that the activities of pathways varied considerably with the conditions of growth (Szynkiewicz *et al.*, 1961; Model and Rittenberg, 1967) and, in part, to the many assumptions made in the calculation of the results.

Many workers have used the ratio of the radioactivity of $^{14}CO_2$ produced by cells grown on [1-^{14}C]glucose to that of cells grown on [6-^{14}C]glucose. The rationale for this lies in the fact that the oxidative pathway will convert the C-1 carbon atom of glucose into CO_2 much faster than the nonoxidative pathway, which must first form methyl-labeled pyruvate and then, after two turns of the citric acid cycle, labeled CO_2. Therefore, during the initial period of growth, [1-^{14}C]-glucose would give rise to labeled CO_2 only *via* the oxidative pathway while [6-^{14}C]glucose would not produce labeled CO_2 by either pathway. Again certain difficulties beset calculations of this type, but despite these, the oxidative pathway has been estimated to operate in the range of 10–30% in *E. coli*. Rittenberg and Ponticorvo (1962) used [1-^{18}O]glucose and [6-^{18}O]glucose in similar experiments, circumventing some of the problems incurred with ^{14}C, and also arrived at a range of 10–30%. Model and Rittenberg (1967) using the specific yields of ^{18}O-labeled CO_2 produced from *E. coli* grown on [1-^{18}O]glucose, [6-^{18}O]glucose, and [1-^{18}O]gluconate, arrived at a value of 24% for the oxidative pathway.

Recently, Nicholson *et al.* (1965) grew *E. coli* on [1-^{18}O]-glucose. The RNA was isolated and was found to contain a relatively high concentration of ^{18}O in the ribose portion of the molecule.

In the experiments described in this paper, *E. coli* was grown on [1-^{18}O]-, [2-^{18}O]-, and [6-^{18}O]glucose and [2-^{18}O]fructose as the sole carbon sources. The growth of the bacteria was terminated in early logarithmic phase. The nucleic acids were isolated and the distribution of ^{18}O in the ribose and deoxyribose moieties was determined. From these data, the relative contributions of the oxidative and nonoxidative pathways have been calculated. Some of the results of these experiments have been published (Caprioli and Rittenberg, 1968a).

The labeling patterns of pentoses derived from the [2-^{18}O]-glucose and [2-^{18}O]fructose experiments suggest that the mechanism of action of the enzyme aldolase in *E. coli* differs from that of the enzyme in mammalian muscle. Preliminary results of some of these experiments have been published (Caprioli and Rittenberg, 1968b).

Materials

All chemicals and enzymes used in the experiments described in this paper were obtained from the Sigma Chemical Co. The $H_2{}^{18}O$ was obtained from the Weizmann Institute of Science, Rehovoth, Israel.

Preparation of [1-^{18}O]Glucose. [1-^{18}O]Glucose was prepared by exchanging D-glucose with high concentration $H_2{}^{18}O$, as previously described (Rittenberg *et al.*, 1961). The [1-^{18}O]-glucose was recrystallized from aqueous ethanol. *Anal.* Calcd: C, 40.00; H, 6.67. Found: C, 39.78; H, 6.93; ^{18}O in glucose (determined by the method of Rittenberg and Ponticorvo, 1956); 10.73 atom % excess (64.4% in the C-1 position). The position of ^{18}O was verified by the synthesis of *N-p*-nitrophenyl-2,3,4,6-tetraacetylglucosylamine (Honeyman, 1963) from the [1-^{18}O]glucose. This derivative, which no longer had the C-1 oxygen atom of the original hexose, contained 0.06 atom % excess ^{18}O. This demonstrated that all the label in [1-^{18}O]glucose was in the C-1 oxygen atom.

Preparation of [6-^{18}O]Glucose. D-Glucose labeled in position 6 was prepared by the method described by Rittenberg *et al.* (1961). *Anal.* Calcd: C, 40.00; H, 6.67. Found: C, 39.30; H, 6.59; 9.21 atom % excess ^{18}O (55.3% in the C-6 oxygen atom). The position of the ^{18}O was verified by the synthesis of the 6-*p*-toluenesulfonyl-1,2,3,4-tetraacetyl derivative (Hardegger and Montavon, 1946) and subsequent elimination of sodium *p*-toluenesulfonate from the molecule on heating with dry NaI in dry acetone at 100° for 2 hr. The dry sodium *p*-toluenesulfonate, containing the oxygen atom originally at the C-6 position of glucose, was analyzed for ^{18}O. This compound contained 18.8 atom % excess ^{18}O (56.4% in a single position). This result proved that all the ^{18}O was originally in the C-6 oxygen atom of glucose.

The [6-^{18}O]glucose was diluted with normal glucose to a final concentration of 8.10 atom % excess ^{18}O (48.6% in the C-6 position) in order to have sufficient material for bacterial growth.

Preparation of [2-^{18}O]Glucose. [2-^{18}O]Glucose was prepared enzymatically as the 6-phosphate ester. About 5 g of barium fructose 6-phosphate was dissolved in 20 ml of high concentration $H_2{}^{18}O$ and incubated for 3 days at 37°. The light amber solution was treated with a small amount of Darco charcoal and filtered. About 5 mg of phosphohexose isomerase, previously dialyzed against distilled water for 24 hr and lyophilized, was added along with a few seed crystals of barium glucose 6-phosphate heptahydrate. After standing at room temperature for 2 days, a crystalline mass formed. The $H_2{}^{18}O$ was recovered by lyophilization. The crystals were suspended in 10 ml of normal cold water, immediately filtered, and thoroughly washed with cold water. The crystals were then dissolved in 50 ml of water by adding a small amount of Dowex 50 (H^+), filtering, and adjusting the pH to 4.5 with dilute NaOH. The solution was passed through a Millipore filter and allowed to stand for 7 days at 37° to exchange any ^{18}O out of the C-1 position. To this solution, 1.2 g of $BaCl_2 \cdot 2H_2O$ was added and the pH was adjusted to 6.8 with dilute NaOH. The solution was kept at 4° for 2 days. The crystals of barium [2-^{18}O]glucose 6-phosphate heptahydrate formed were filtered, washed with cold water, and air dried; yield 2.3 g. *Anal.* Calcd: C, 13.77; H, 4.97. Found: C, 13.86; H, 4.89; 5.39 atom % excess ^{18}O. Enzymatic analysis (Horecker

and Wood, 1957) of the compound showed it to be greater than 98% pure.

The position of the ^{18}O was verified by synthesis of several derivatives. The 2,4-dintrophenylosazone derivative (Robison and King, 1931) contained 0.02 atom % excess ^{18}O, showing that only the C-1 and C-2 oxygen atoms of the original glucose could have contained ^{18}O. *N-p*-Nitrophenyl-2,3,4,6-tetraacetylglucosylamine was prepared from barium [2-^{18}O]-glucose 6-phosphate·$7H_2O$ by converting the latter into the sodium form and incubating it with potato acid phosphatase in 0.001 M citric acid buffer at pH 4.8 at 37°. After the reaction was completed, the [2-^{18}O]glucose was desalted by passing it through Dowex 1-X8 (OH^-) and Dowex 50-X8 (H^+). The amine derivative was then prepared as before. Analysis of the amine showed it to contain 6.83 atom % excess ^{18}O (75.1 in the C-2 oxygen atom).

For the growth of *E. coli*, the labeled hexose phosphate was diluted twofold with normal barium glucose 6-phosphate·$7H_2O$. The final isotope concentration was 2.68 atom % ^{18}O (37.6% in the C-2 oxygen atom); 2 g of barium [2-^{18}O]glucose 6-phosphate·$7H_2O$ was dissolved in 10 ml of minimal medium with the aid of 2 N HCl, and a stoichiometric amount of Na_2SO_4 was added. The solution was filtered, adjusted to pH 7.0, and sterilized by Millipore filtration. The solution was then added to 1 l. of sterile minimal media for the growth of the bacteria, as described in the Methods section.

Preparation of [2-^{18}O]Fructose. [2-^{18}O]Fructose was prepared by exchanging 10 g of recrystallized fructose with 10 ml of high concentration $H_2{}^{18}O$, containing 0.1 ml of concentrated HCl, at 40° for 4 days. The water was recovered by lyophilization. The fructose was dissolved in a minimum amount of normal water, absolute alcohol was added, and the solution was kept at 4°. After several days, crystals formed and were removed by filtration. The crystals were washed with cold absolute alcohol and dried over P_2O_5 *in vacuo*. The yield was 9.3 g. *Anal.* Calcd: C, 40.00; H, 6.67. Found: C, 40.13; H, 6.96; 6.53 atom % excess ^{18}O (39.2% in the C-2 position).

The position of the ^{18}O was verified by preparing the 2,4-dinitrophenylhydrazone–pyridine solvate derivative (White and Secor, 1963). This compound, whose C-2 oxygen atom was lost in the formation of the hydrazone, contained 0.003 atom % excess ^{18}O, demonstrating that all of the label was originally in the C-2 position of fructose.

Preparation of [5′-^{18}O]-2′,3′-Isopropylideneuridine. To 200 mg of 5′-*p*-toluenesulfonyl-2′,3′-isopropylideneuridine (Todd, 1957) was added 5 ml of dry acetone, 0.1 ml of high concentration [^{18}O]acetic acid, and 40 mg of NaOH. The mixture was refluxed overnight. The acetone was removed and the solution was extracted with chloroform. The chloroform extract was washed with 3 N H_2SO_4, a saturated solution of $NaHCO_3$, H_2O, and was dried over Na_2SO_4. The volume was reduced to about 0.5 ml and petroleum ether (bp 30–60°) was added. A precipitate formed immediately. The product, 5′-acetyl-2′,3′-isopropylideneuridine, contained 3.64 atom % excess ^{18}O; yield approximately 120 mg.

Above 100 mg of the above product was dissolved in 0.1 M NaOH in 90% ethanol and allowed to stand at room temperature for several days. The ethanol was removed and the aqueous solution was neutralized and lyophilized. The solid was extracted with chloroform and the product, 2′,3′-isopropylideneuridine, was precipitated with petroleum ether. On paper chromatography, the product gave only one ultraviolet-absorbing spot whose R_F value was identical with that of commercially obtained material; yield about 50 mg. *Anal.* Calcd: C, 50.63; H, 5.63: Found: C, 50.49; H, 5.68; 1.97 atom % excess ^{18}O (11.8% in the 5′-oxygen atom).

Methods

Growth of Bacteria. The C salts medium of Roberts *et al.* (1957) was used for the growth of *E. coli* strain B. The medium contained a limiting amount of glucose (0.7 mg/ml). A stock culture was grown twice on normal hexose and finally once on labeled hexose; the latter being used for the inoculation of the large cultures. Exactly 10 ml of the inoculation culture was added to each liter of medium containing labeled hexose. In a typical experiment, 5 l. of bacteria was grown for approximately 5 hr at 37° with rapid shaking to a Klett optical density of 90 (660-mμ filter). At this point, the cells were in midexponential phase, corresponding to approximately 6×10^8 cells/ml. The cells were immediately chilled, harvested, and washed with 0.9% NaCl solution. The average yield of bacteria was about 0.9 g/l. wet weight.

Isolation of Nucleic Acids. To 5 g of frozen cells, there was added 10 ml of cold 0.001 M Tris buffer (pH 7.2) containing 0.01 M magnesium acetate and 2.5% sodium lauryl sulfate. The mixture was homogenized in an ice-cold Waring blender at low speed for about 1 min. The creamy suspension was immediately transferred to a flask containing an equal volume of redistilled phenol saturated with water and vigorously shaken for 2 hr at room temperature. The suspension was centrifuged at 10,000*g* for 30 min and the aqueous layer was removed. The phenol and protein layers were reextracted with 10 ml of 0.001 M Tris–0.01 M magnesium acetate buffer (pH 7.2). To the combined aqueous layers was added one-tenth volume of 20% potassium acetate solution (previously adjusted to pH 5.0 with glacial acetic acid). Three volumes of cold 95% ethanol was added and the mixture was placed in the freezer at −20° overnight. The nucleic acids were then collected by centrifugation in the cold at 15,000*g* for 30 min. The precipitate was washed once with cold 70% ethanol and recentrifuged. The RNA–DNA mixture was dissolved in a small amount of distilled water and dialyzed for 24 hr against distilled water. The dialyzed solution was then lyophilized. The product was a mixture of RNA and DNA, containing small amounts of polysaccharides and proteins.

Degradation of Nucleic Acids. The crude RNA–DNA mixture was hydrolyzed by the combined action of snake venom phosphodiesterase and 5′-nucleotidase (both from *Crotalus adamanteus* venom). Phosphodiesterase is known to hydrolyze the 3′-phosphate ester linkage with cleavage between the phosphorous and oxygen atoms (Dixon and Webb, 1964). 5′-Nucleotidase hydrolyzes nucleoside 5′-phosphate esters also with P–O cleavage (Koshland and Springhorn, 1956). The combination of these two enzymes allows the resulting nucleosides to retain both of its original bridge oxygen atoms.

In a typical reaction, 100 mg of the RNA–DNA mixture was dissolved in 50 ml of 0.01 M Tris buffer (pH 8.9) at 37°, containing 0.01 M magnesium chloride. To this solution was added 5–10 mg each of phosphodiesterase and nucleotidase. About 3 mg of penicillin G and 6 mg of streptomycin sulfate were added to retard bacterial growth. The solution was incubated at 37° with shaking for about 30–40 hr. The reaction

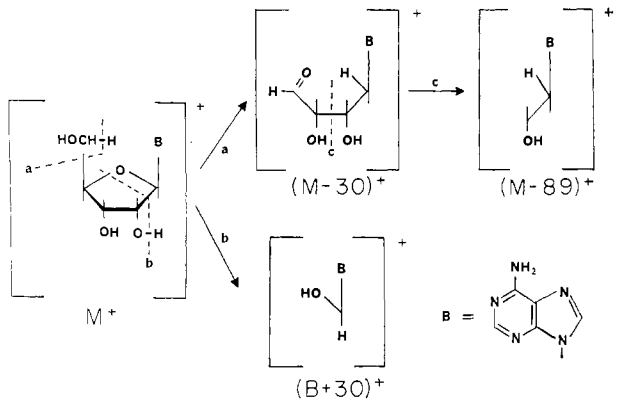

FIGURE 1: Partial fragmentation of adenosine.

was followed by determination of the inorganic phosphate liberated, according to Lowry and Lopez (1946). When the reaction was completed, the mixture was lyophilized.

Separation of Nucleosides. The lyophilized mixture was suspended in 10 ml of 30% aqueous methanol and loaded on an AG-1-X2(OH^-) column (1.5 × 45 cm) which had been washed thoroughly with 2 N NaOH, distilled water, and finally equilibrated with 30% methanol (Dekker, 1965). The column was eluted with 30% methanol and the effluent was monitored with Isco Model UV2 ultraviolet analyzer at 254 mμ.

After deoxycytidine, deoxyadenosine, and cytidine had emerged, the eluent was changed to 60% aqueous methanol in order to elute adenosine. The remaining nucleosides were washed from the column in a single peak (consisting mainly of uridine and guanosine) with 0.01 M $(NH_4)HCO_3$. The various fractions were pooled, concentrated to a small volume on a flash evaporator, and lyophilized. Uridine and guanosine were separated from the last fraction by column chromatography on Sephadex G-10 (1.5 × 45 cm) eluting with water (Dirheimer and Ebel, 1967). In this manner, the major nucleosides were isolated substantially free of salts or other contaminants.

The nucleosides were chromatographed on Whatman No. 1 chromatography paper using ethanol–*t*-butyl alcohol–water–formic acid (12:4:3:1, v/v) as a solvent. In all cases, the R_F values of the samples were identical with those of authentic nucleosides. In addition, the ultraviolet spectra taken with a Cary Model 14 spectrophotometer were identical with those reported in the literature.

Control Experiment. A control was carried out to determine whether the degradation and isolation techniques employed would lead to isotope exchange. Unlabeled RNA from *E. coli* was enzymatically hydrolyzed in 1.473 atom % excess $H_2{}^{18}O$ under the usual conditions. The nucleosides were isolated and analyzed for ^{18}O by the combustion technique. Adenosine and cytidine each contained less than 0.002 atom % excess ^{18}O, demonstrating that the procedures employed did not lead to ^{18}O exchange with the medium.

Analysis of Nucleosides for ^{18}O Distribution. The distribution of ^{18}O in the ribose and deoxyribose portion of the nucleosides was determined by mass spectrometry. Under bombardment by an electron beam in a mass spectrometer, the vaporized nucleoside molecule acquires a positive charge through the loss of an electron. The major portion of these ions undergoes fragmentation to small positively charged ions and neutral fragments due to the large kinetic energy which has been imparted to them. The intensities and masses of all the charged ions are then recorded by the analyzer portion of the spectrometer.

The work of Biemann and McCloskey (1962), Hanessian *et al.* (1966), and McCloskey *et al.* (1968) has shown that the mass spectrum of a nucleoside contains fragment ions whose oxygen atoms are known to arise from specific positions of the pentose portion of the molecule. The concentration of ^{18}O in a fragment ion can be calculated from the intensities of the various isotope peaks. A peak having mass X will show an isotope peak at mass $X + 2$ due to the abundance of one ^{18}O atom or two ^{13}C atoms (the contribution of ^2H and ^{15}N to this peak is negligible). The intensity of the $X + 2$ peak due to the natural abundance of the various isotopes can be calculated (Beynon, 1960) or measured directly from the spectrum of an unlabeled nucleoside. In the latter case, the atom % excess ^{18}O in a fragment ion can be determined using

$$\text{atom \% excess } {}^{18}O = \frac{h^s_{X+2} - h^s_X\,(h^n_{X+2}/h^n_X)}{h^s_X - h^s_{X+2} - h^s_X\,(h^n_{X+2}/h^n_X)} \times 100$$

where h_X = intensity of peak having mass X, h_{X+2} = intensity of second isotope peak having mass $X + 2$, s = sample (labeled) compound, and n = unlabeled compound.

In the mass spectrum of adenosine, the molecular ion, M^+, appears at *m/e* 267. The intensities of the M^+ and $(M + 2)^+$ peaks can be used to calculate the concentration of ^{18}O in this ion, *i.e.*, in the 2′ + 3′ + 4′ + 5′ positions. The molecular ion can fragment in several ways, one of which leads to the formation of an $(M - 30)^+$ ion through the elimination of 5′-carbon and oxygen atoms as formaldehyde (see Figure 1, fragmentation a). The intensities of the $(M - 30)^+$ and $[(M - 30) + 2]^+$ ions can be used to calculate the concentration of ^{18}O in the 2′ + 3′ + 4′-oxygen atoms. The $(M - 30)^+$ ion can fragment to form an $(M - 89)^+$ ion which contains only the 2′-oxygen atom of the original molecule (see Figure 1, fragmentation c). The molecular ion can also yield, through a rearrangement process, a $(B + 30)^+$ (B = mass of adenyl group) fragment ion which contains only the 4′-oxygen atom (see Figure 1, fragmentation b). Isotope in the 3′ position can be measured by difference. In this way ^{18}O can be measured in all positions of the pentose portion of adenosine.

Deoxyadenosine and deoxycytidine show peaks in their mass spectra at M^+, $(M - 30)^+$, and $(B + 30)^+$. Uridine differs from the other nucleosides by eliminating the entire primary alcohol group from M^+ to yield an $(M - 31)^+$ ion. However, it does give fragments at $(M - 89)^+$ and $(B + 30)^+$ corresponding to those of the other nucleosides.

Cytidine and guanosine were not sufficiently volatile to give good mass spectra. However, their trimethylsilyl (Me_3Si) derivatives were quite volatile and were easily prepared (McCloskey *et al.*, 1968). Uridine was also analyzed as its Me_3Si derivative when the sample size was small. Approximately 1 mg of dry sample was placed in about 0.2 ml of Tri-Sil (Pierce Chemical Co. brand of bistrimethylsilylacetamide–trimethylchlorosilane in pyridine) and warmed to 60° for about 1 min. After 1 hr at room temperature, the solution was transferred into melting point capillary tubes with a syringe and the sol-

vent was removed on a high vacuum line. The capillary tube was then placed into the direct probe of the mass spectrometer.

Guanosine-$(Me_3Si)_5$ shows a large M^+ ion at *m/e* 643. This ion contains all four ribose oxygen atoms (see Figure 2). The M^+ ion can fragment to lose the base moiety, a hydrogen atom, and the trimethylsilated primary alcohol group to form an $(S - 104)^+$ ion (S = mass of trimethylsilated ribosyl group) at *m/e* 245. This ion contains the 2′-, 3′-, and 4′-oxygen atoms of guanosine. The molecular ion can also yield at $(B' + 30)^+$ fragment (B′ = guanyl-$(Me_3Si)_2$ group) at *m/e* 324 which contains only the 4′-oxygen atom and also a $(B' + 116)^+$ fragment at *m/e* 410 containing only the 2′-oxygen atom. In this way ^{18}O in the 2′, 4′, and 5′ positions can be measured directly.

The mass spectrum of trimethylsilylcytidine exhibits peaks of both the tri- and tetratrimethylsilated forms, with peaks at $(S - 104)^+$, $(B'' + 30)^+$, and $(B'' + 116)^+$ (B″ = cytosyl-$(Me_3Si)_1$ group) for the $(Me_3Si)_4$ derivatives and M^+ and $(M - 15)^+$, as well as the others, for the $(Me_3Si)_3$ derivative. The $(M - 15)^+$ fragment is formed through the loss of a methyl group from one of the trimethylsilyl functions. The fragments formed from cytidine-$(Me_3Si)_3$ and also those from uridine-$(Me_3Si)_3$ are produced by fragmentations similar to those described for guanosine-$(Me_3Si)_5$.

In a few cases, *i.e.*, for some ions of cytidine-$(Me_3Si)_3$ and uridine-$(Me_3Si)_3$, interfering ions prevented the measurement of the $X + 2$ peaks for ^{18}O abundance calculations.

The mass spectra were principally obtained from a CEC-21-110C double-focusing mass spectrometer. The samples were introduced by a direct probe and were run at an ionizing energy of 70 eV and a block temperature of about 150°. For peak intensity measurements, a resolution of 1000 was used.

For the free nucleosides, the experimental error in the measurements of the abundances of ^{18}O was approximately ±0.5 atom %. In the case of the trimethylsilylnucleosides, the error was greater, ±1.5 atom %, due to the complexity of the spectra and also to the relatively large isotope corrections for the natural abundances of ^{29}Si (4.71%) and ^{30}Si (3.12%). The peak intensities used in the calculation of ^{18}O abundances are average values obtained in most cases from a minimum of five different spectra.

To test the accuracy of this method for the determination of ^{18}O, synthetically prepared [5′-^{18}O]2′,3′-isopropylideneuridine was analyzed. In the mass spectrum of this compound, the molecular ion is of low intensity. However, the ion at $(M - 15)^+$, formed through the loss of a methyl group from M^+, is large. The ion at $(M - 31)^+$ is formed through the loss of the primary alcohol group from M^+. Measurement of the $(M - 15)^+$ and $(M - 31)^+$ ions showed that there was 11.9 atom % excess ^{18}O in the 5′-oxygen atom. This answer was in good agreement with that obtained by the combustion technique showing 1.97 atom % excess ^{18}O in the molecule (11.8% in a single position).

Results

Verification of Elemental Composition of Fragments. High-resolution mass spectra of the labeled nucleosides were obtained to verify that the peaks being measured were in fact the proper isotope peaks and were not due to other ions having the same nominal mass. The exact mass of the ions was determined using perfluorokerosene (Penninsular Chemical Co.) as a mass standard. This allows the elemental composition of each ion to be determined. In all cases, only a single species of ions was present at a particular mass and these had the expected elemental composition. For example, Table I shows both the measured and calculated masses for some of the ^{18}O isotope peaks of adenosine and deoxyadenosine derived from the [1-^{18}O]glucose experiment. Similar determinations were made for the other nucleosides.

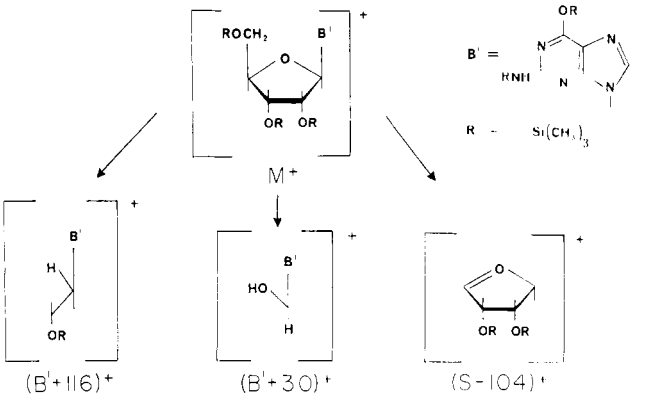

FIGURE 2: Partial fragmentation of pentakis(trimethylsilyl)-guanosine.

Mass Spectra of Normal (Unlabeled) Nucleosides. The mass spectra of normal nucleosides were obtained and the peak heights of some of the fragments were measured in order to calculate values corresponding to natural isotopic abundance.

The intensity of peak $X + 2$ in the case of the unlabeled nucleosides is a result of the presence of naturally abundant ^{18}O and ^{13}C. For the trimethylsilane derivatives, ^{29}Si and ^{30}Si also contribute to this peak.

Nucleosides from E. coli Grown on [1-^{18}O]Glucose. The C-1 oxygen atom of glucose exchanges slowly with an oxygen atom in the water of the medium. Rittenberg and Graff (1958) have calculated from their data that the loss of ^{18}O from [1-^{18}O]glucose, in an aqueous medium at 37° at pH 7, is approximately 2.2%/hr. The original concentration of ^{18}O in the glucose before addition to the medium was 64.4 atom % excess in the C-1 position. Using the above rate of exchange, the average ^{18}O content of the glucose metabolized by the bacteria during a 5-hr growth period was 57.3 atom % excess in the C-1 position.

The results of the calculations of ^{18}O abundances are shown in Table II. Adenosine and deoxyadenosine will be considered first since these two nucleosides contain oxygen atoms only in the pentose moiety. The molecular ion of adenosine, containing the 2′-, 3′-, 4′-, and 5′-oxygen atoms, showed a total of 20 atom % excess ^{18}O. The $(M - 30)^+$ ion, containing the 2′-, 3′-, and 4′-oxygen atoms, had no significant isotope peak above natural abundance at $[(M - 30) + 2]^+$. Neither the $(M - 89)^+$ ion nor the $(B + 30)^+$ ion contained excess ^{18}O. This demonstrated that all of the ^{18}O was in the 5′-oxygen atom of adenosine. Exactly the same result was obtained for deoxyadenosine and deoxycytidine, *i.e.*, all of the 20 atom % excess ^{18}O present in M^+ was lost in the formation of $(M - 30)^+$ fragment.

TABLE I: Exact Masses of Some ^{18}O Isotope Peaks in Nucleoside Spectra.

Compound	Peak	Elemental Composition	Theoretical Mass[a]	Measured Mass
Adenosine	$(M + 2)^+$	$C_{10}H_{13}N_5O_3{}^{18}O$	269.1010	269.1021
	$([M - 30] + 2)^+$	$C_9H_{11}N_5O_2{}^{18}O$	239.0904	239.0906
	$([M - 89] + 2)^+$	$C_7H_8N_5{}^{18}O$	180.0771	180.0775
	$([B + 30] + 2)^+$	$C_6H_6N_5{}^{18}O$	166.0614	166.0612
Deoxyadenosine	$(M + 2)^+$	$C_{10}H_{13}N_5O_2{}^{18}O$	253.1061	253.1080
	$([M - 30] + 2)^+$	$C_9H_{11}N_5O^{18}O$	223.0953	223.0964
	$([B + 30] + 2)^+$	$C_6H_6N_5{}^{18}O$	166.0614	166.0607

[a] Calculated using $^{12}C = 12.0000$ amu.

TABLE II: ^{18}O Abundance of Ions in Mass Spectra of Nucleosides.

Nucleoside	Peak X	Oxygen Atom(s) Present in Ion	^{18}O Concentration in Ions (Atoms % Excess) [1-^{18}O]-Glucose Expt	[6-^{18}O]-Glucose Expt	[2-^{18}O]-Glucose Expt	[2-^{18}O]-Fructose Expt
Adenosine	M^+	2′ + 3′ + 4′ + 5′	20.2	31.8	11.3	13.7
	$(M - 30)^+$	2′ + 3′ + 4′	0.3	0.6	11.8	13.6
	$(M - 89)^+$	2′	0.4	0.7	4.8	4.5
	$(B + 30)^+$	4′	0.0	0.3	6.6	9.4
Deoxyadenosine	M^+	3′ + 4′ + 5′	20.0	31.8	7.1	9.3
	$(M - 30)^+$	3′ + 4′	0.0	0.3	6.7	9.9
	$(B + 30)^+$	4′	0.4	0.6	7.2	9.8
Guanosine $(Me_3Si)_5$	M^+	2′ + 3′ + 4′ + 5′	20.7	32.4	11.7	14.6
	$(B' + 116)^+$	2′	0.0	2.8	...	4.4
	$(B' + 30)^+$	4′	0.0	1.6	8.5	8.0
	$(S - 104)^+$	2′ + 3′ + 4′	0.0	0.7	11.9	15.4
Uridine $(Me_3Si)_3$	$(M - 15)^+$	2′ + 3′ + 4′ + 5′	19.3	32.1	11.1	13.6
	$(S - 104)^+$	2′ + 3′ + 4′	0.0	2.5	12.2	14.8
Cytidine $(Me_3Si)_3$	$(M - 15)^+$	2′ + 3′ + 4′ + 5′	19.2	29.6	10.0	14.2
	$(S - 104)^+$	2′ + 3′ + 4′	0.0	2.1	10.5	15.2
	$(B'' + 30)^+$	4′	0.0	0.7		10.6
Deoxycytidine	M^+	3′ + 4′ + 5′	20.2			
	$(M - 30)^+$	3′ + 4′	0.6			

The other nucleosides were introduced into the mass spectrometer as their trimethylsilane derivatives. The values obtained for these compounds were in agreement with those of the free nucleosides. Guanosine-$(Me_3Si)_5$ showed a large molecular ion at *m/e* 643. The intensity of the ion at *m/e* 645 gave a measure of the total concentration of ^{18}O in the molecule. Formation of the $(S - 104)^+$ ion at *m/e* 245 resulted in the decrease in the intensity of the ^{18}O isotope peak at *m/e* 247 to the level of natural abundance. This showed that all the ^{18}O was in the 5′-oxygen atom.

A similar examination of cytidine-$(Me_3Si)_3$ and uridine-$(Me_3Si)_3$, *i.e.*, measurement of $(M - 15)^+$ and $(S - 104)^+$ ions together with their isotope peaks, showed them to be labeled with about 19 atom % excess ^{18}O in the 5′ position. None of the other oxygen atoms in these nucleosides contained excess ^{18}O.

Nucleosides from E. coli Grown on [6-^{18}O]Glucose. Adenosine and deoxyadenosine, isolated from bacteria grown on [6-^{18}O]glucose, were analyzed as the free nucleosides while cytidine, guanosine, and uridine were analyzed as their trimethylsilane derivatives. The mass spectra are shown in Figure 3 and the results of the ^{18}O abundance calculations in Table II. All the nucleosides were found to be labeled almost entirely in the 5′-oxygen atom. The ^{18}O concentration in the metabolized glucose was 48.6 atom % excess in the C-6 oxygen atom: the ^{18}O concentration in the isolated nucleosides was 31 atom % excess in the 5′-oxygen atom. Both the oxygen atoms present in the bases and those in the secondary hy-

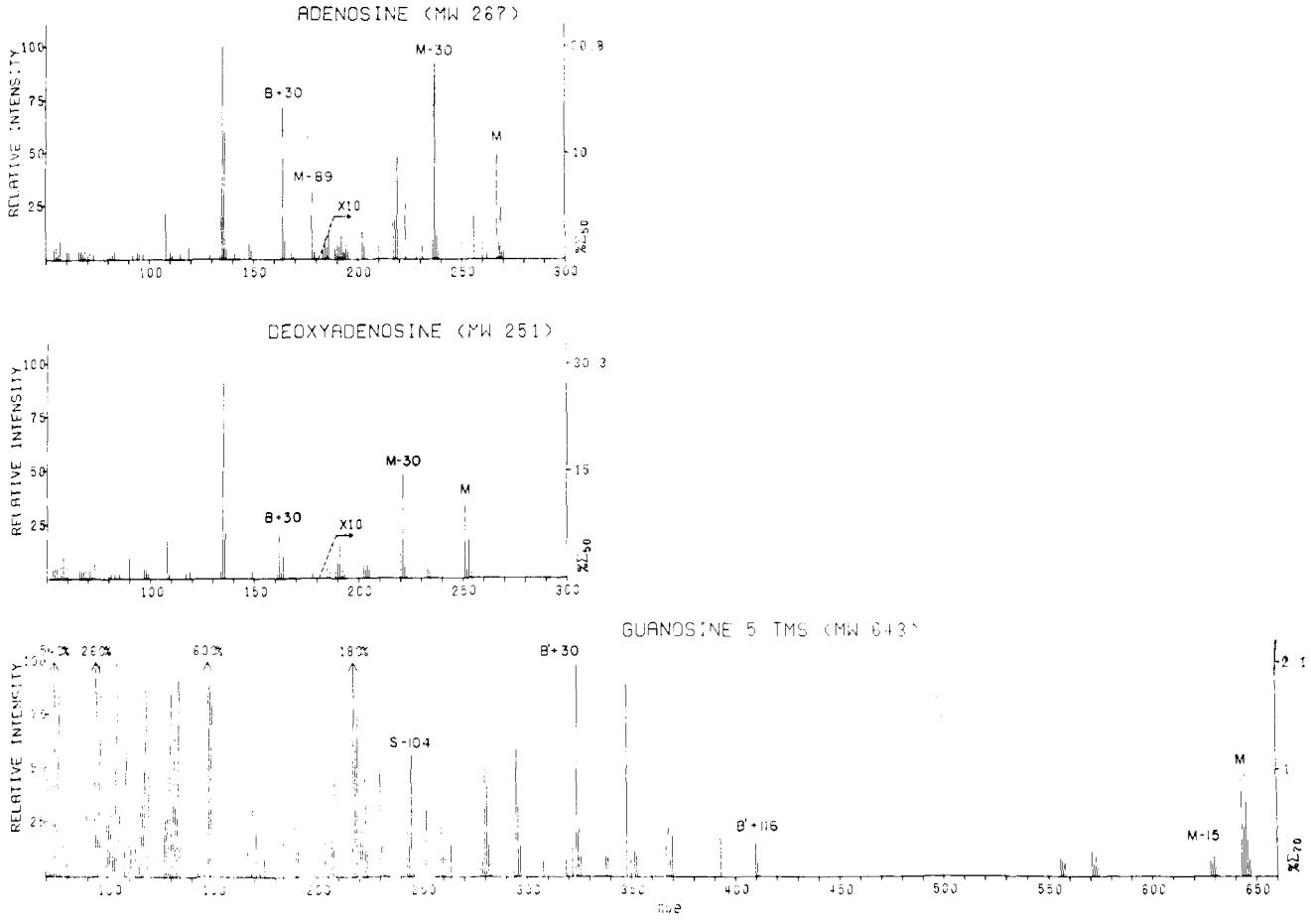

FIGURE 3: Mass spectra of nucleosides from [6-^{18}O]glucose.

droxyl groups of the pentoses did not contain significant concentrations of ^{18}O.

Nucleosides from E. coli Grown on [2-^{18}O]Glucose 6-Phosphate. E. coli was grown on [2-^{18}O]glucose 6-phosphate as the sole carbon source. Glucose 6-phosphate has been shown to enter the bacterial cell intact (Fraenkel *et al.*, 1964). This eliminates the possibility of ^{18}O exchange through the hydrolysis and rephosphorylation of the molecule. Since the glucose 6-phosphate is common to both the oxidative and nonoxidative pathways, the bacteria were grown directly on this derivative. The nucleosides were isolated and analyzed as before.

The results of the ^{18}O abundance calculations are given in Table II. Both the M^+ and $(M - 30)^+$ ions of adenosine contained approximately the same concentration of ^{18}O (11.5 atom % excess) showing that none of the label was present in the 5′-oxygen atom. The $(M - 89)^+$ ion, which contained only the 2′-oxygen atom, had 5 atom % excess ^{18}O while the $(B + 30)^+$ ion, which contained only the 4′-oxygen atom, had 7 atom % excess ^{18}O. By difference, there was no ^{18}O in the 3′-oxygen atom. Thus, ^{18}O was present only in the 2′- and 4′-oxygen atoms.

The M^+, $(M - 30)^+$, and $(B + 30)^+$ ions of deoxyadenosine all contained about 7 atom % excess ^{18}O. Thus, only the 4′-oxygen atom of deoxyadenosine contained label with a concentration equal to that of the 4′-oxygen atom of adenosine. The other nucleosides also showed approximately the same distribution of ^{18}O.

Nucleosides from E. coli Grown on [2-^{18}O]Fructose. The keto oxygen atom of fructose exchanges with an oxygen atom of the water in the medium at the rate of approximately 1.4%/hr at 37° at pH 7.0 (Model *et al.*, 1968). The average ^{18}O concentration in the metabolized fructose, starting with 39.2 atom % excess ^{18}O, was calculated to be 36.5 atom % excess ^{18}O in the C-2 oxygen atom after correcting for this exchange.

The mass spectra are shown in Figure 4 and the results of the ^{18}O calculations in Table II. Adenosine contained a total of 14 atom % excess ^{18}O. No ^{18}O was present in the the 5′-oxygen atom, since the $(M - 30)^+$ ion contained a concentration of label equal to that of the molecular ion. Examination of the $(M - 89)^+$ and $(B + 30)^+$ ions showed that there was about 5 atom % excess ^{18}O in the 2′-oxygen atom and 9 atom % excess ^{18}O in the 4′-oxygen atom. There was no ^{18}O in the 3′ position. Deoxyadenosine contained ^{18}O only in the 4′-oxygen atom of abundance equal to that of the same position of adenosine. The other nucleosides showed similar labeling patterns.

Discussion

Glucose metabolized exclusively by way of the oxidative pathway would be converted into ribulose 5-phosphate through

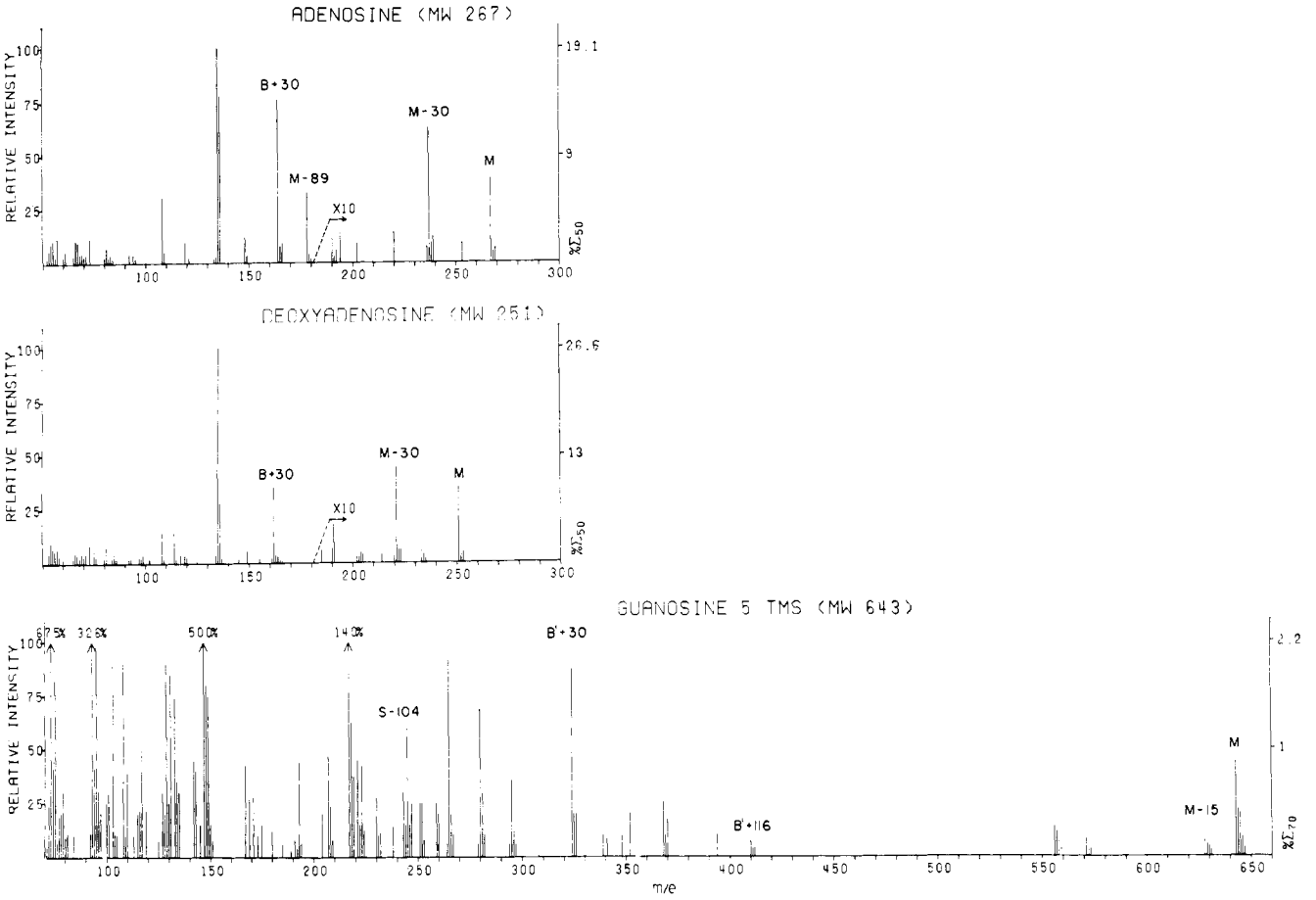

FIGURE 4: Mass spectra of nucleosides from [2-^{18}O]fructose.

the loss of the C-1 carbon atom as carbon dioxide. Positions C-2 through C-6 of glucose would become positions C-1 through C-5 of pentose. Therefore, [1-^{18}O]glucose could not give rise to labeled ribose. However, [2-^{18}O]glucose would yield [1-^{18}O]ribose and [6-^{18}O]glucose would yield [5-^{18}O]ribose. ^{18}O appearing in the C-1 oxygen atom of ribose 5-phosphate would not appear in the nucleosides since this oxygen atom is lost in the formation of glycosidic linkage with the base. Thus, only [6-^{18}O]glucose would produce a nucleoside labeled in the pentose moiety *via* the oxidative pathway. The concentration of ^{18}O in the C-5 oxygen atom of the pentose would be equal to that of the C-6 oxygen atom of the original glucose. The data are clearly inconsistent with the above observations.

If glucose were metabolized exclusively *via* the nonoxidative pathway, the labeling pattern of the pentoses would be quite different. The cleavage of fructose 1,6-diphosphate by aldolase produces dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. These two triose phosphates are in isotopic equilibrium due to the rapid, reversible action of the enzyme triosephosphate isomerase. Thus, both [1-^{18}O]-glucose and [6-^{18}O]glucose would give rise to [3-^{18}O]glyceraldehyde 3-phosphate containing one-half the initial ^{18}O concentration of the hexose. [2-^{18}O]Glucose would form [2-^{18}O]-glyceraldehyde 3-phosphate with one-half the original concentration of label, provided that the actions of triose phosphate isomerase and aldolase do not produce ^{18}O exchange. The mechanism of the isomerase reaction has been shown (Rieder and Rose, 1959) to involve an enzyme-bound enolate anion and would not lead to exchange. Evidence is presented later which shows that aldolase of *E. coli* does not result in an obligatory loss of the C-2 oxygen atom. Therefore, the transfer of an active glycolaldehyde group from a 2-keto sugar phosphate to [^{18}O]-glyceraldehyde 3-phosphate by transketolase would produce labeled ribose phosphate. [1-^{18}O]- and [6-^{18}O]-glucose would thus give rise to [5-^{18}O]ribose 5-phosphate and [2-^{18}O]glucose to [4-^{18}O]ribose 5-phosphate. In the latter case, the resulting pentose may also be labeled in the C-2 oxygen atom. The concentration in this position would depend upon the extent to which [2-^{18}O]fructose 6-phosphate, or other suitable 2-keto sugar, acted as an active glycolaldehyde donor. Thus, the C-2 oxygen atom of the ribose could contain ^{18}O in a concentration varying from zero up to the total concentration of the original hexose.

The results of the experiments described in this paper are summarized in Table III. When *E. coli* was grown on [1-^{18}O]-glucose (containing 57.3 atom % excess ^{18}O in the C-1 position), the ribose and deoxyribose portion of the nucleic acids contained about 20 atom % excess ^{18}O in the 5′ position. No other oxygen atoms of the nucleosides were labeled. These data are consistent with the evidence suggesting that both the oxidative and nonoxidative pathways contribute to the formation of pentose in the nucleic acids. The oxidative pathway would produce an unlabeled pentose; the nonoxidative pathway would produce a pentose with 28.7 atom % excess ^{18}O

in the 5′-oxygen atom. This shows that 30% of the pentose in the nucleic acids arose *via* the oxidative pathway and 70% *via* the nonoxidative pathway.

Growth of *E. coli* on [6-^{18}O]glucose (containing 48.6 atom % excess ^{18}O in the C-6 oxygen atom) produced a pentose which had 31 atom % excess ^{18}O in the C-5 position. No other oxygen atoms contained excess label. The oxidative pathway would produce a 5′-labeled nucleoside containing 48.6 atom % excess ^{18}O while the nonoxidative pathway would produce a 5′-labeled nucleoside containing 24.3 atom % excess ^{18}O. The experimental data show that 28% of the pentose in the nucleic acids arose *via* the oxidative pathway and 72% *via* the nonoxidative pathway. These results agree well with that obtained from the [1-^{18}O]glucose experiment.

In this discussion, it has been assumed that the isomerization of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate catalyzed by triosephosphate isomerase leads to complete isotopic equilibrium. The ^{18}O distribution in the nucleosides from the [1-^{18}O]- and [6-^{18}O]glucose experiments lends support to this assumption, *i.e.*, the calculations of the participation of the pathways are the same in both cases. Since glyceraldehyde 3-phosphate (originally formed from C-4, C-5, and C-6 of glucose) is the C_2 acceptor, any significant deviation from the equilibrium condition would lead to a large change in the concentration of ^{18}O in the nucleosides and, therefore, a large difference in the calculations of the participation of the pathways. This was not found. This observation is also true for the [2-^{18}O]hexose experiments discussed in the following paragraphs.

When [2-^{18}O]glucose containing 37 atom % excess ^{18}O was used as the sole carbon source for the growth of *E. coli*, the nucleosides isolated from the nucleic acids contained about 5 atom % excess ^{18}O in the 2′ position and 7 atom % excess in the 4′ position. Neither the 3′- nor the 5′-oxygen atom was labeled. This is qualitatively consistent with the results obtained from the previous experiments. The oxidative pathway would not produce a labeled nucleoside, while the nonoxidative pathway would produce a [2′,4′-^{18}O]nucleoside through the formation of [2-^{18}O]glyceraldehyde 3-phosphate. However, quantitatively, these results are more difficult to explain. A 3:7 ratio for the contribution of the oxidative:nonoxidative pathways observed in the previous experiments would lead to labeling of the C-4 oxygen atom with about 12 atom % excess ^{18}O and the C-2 oxygen atom with 0–26 atom % excess ^{18}O. Experimentally, about 60% of the label expected in the C-4 position was actually found there. This discrepancy may be explained in several ways. The most likely, in view of the other experiments, is that the lifetime of [2-^{18}O]dihydroxyacetone phosphate is sufficiently large to allow for the exchange of the C-2 oxygen atom with the medium. Experiments by Model *et al.* (1968) have shown that the half-life of the nonenzymatic exchange of [2-^{18}O]dihydroxyacetone phosphate is less than 1 min. This exchange would result in a lower concentration of ^{18}O in the 4′-oxygen atom of the nucleosides than expected.

E. coli which was grown on [2-^{18}O]fructose containing 36 atom % excess ^{18}O formed ribose which contained about 14 atom % excess ^{18}O. Approximately 5 atom % excess ^{18}O was in the 2′-oxygen atom and 9 atom % excess in the 4′-oxygen atom of the nucleosides. Although this labeling pattern is similar to that obtained from the [2-^{18}O]glucose experiment, there is a difference in the ^{18}O concentration of the 4′-oxygen atom. The 4′-oxygen atom of the nucleosides derived from [2-^{18}O]glucose 6-phosphate contained about 7 atom % excess ^{18}O while that derived from [2-^{18}O]fructose contained about 9 atom % excess ^{18}O. From what is known about the metabolism of these two hexoses, it is not apparent why this difference should have occurred. This could be a result of the difference in the utilization of glucose 6-phosphate and fructose. However, in view of the purpose and scope of these experiments, this discrepancy is not large. Thus, these two results may be interpreted in a similar manner.

TABLE III: Summary of ^{18}O Distributions in the Nucleosides.

Position of Oxygen Atom	^{18}O Concentration (Atom % Excess)[a]			
	[1-^{18}O]-Glucose[b] Expt	[6-^{18}O]-Glucose[c] Expt	[2-^{18}O]-Glucose[d] Expt	[2-^{18}O]-Fructose[e] Expt
2′	0	1	5	5
3′	0	0	0	0
4′	0	1	7	9
5′	20	31	0	0

[a] Average value. [b] 57.3 atom % excess ^{18}O. [c] 48.6 atom % excess ^{18}O. [d] 37.6 atom % excess ^{18}O. [e] 36.5 atom % excess ^{18}O.

In addition to the reactions previously discussed, there are several which could lead to a redistribution of the ^{18}O label by the re-formation of hexose phosphate. Randomization could take place through reactions catalyzed by transketolase and transaldolase. This would result in the labeling of the C-1 and C-3 positions of hexose starting with C-2-labeled hexose and also of the C-3 position starting from C-1-labeled hexose. However, it was found in these experiments that neither the ribose obtained from *E. coli* grown on [1-^{18}O]glucose nor that obtained from [2-^{18}O]glucose contained ^{18}O in the 3′ position. This shows that little, if any, randomization occurred *via* the transketolase–transaldolase reactions. This is in agreement with the results of other workers who studied the ^{14}C distribution of the glucose of glycogen isolated from *E. coli* grown in the exponential phase on [2-^{14}C]glucose (Szynkiewicz *et al.*, 1961; Sable and Cassisi, 1962). The small redistribution of ^{14}C suggested that there was only 1–2% randomization of the hexose carbon skeleton. In addition, these results as well as that of other works (Model and Rittenberg, 1967) show that the resynthesis of hexose phosphate from triose phosphate *via* the enzyme aldolase does not occur to any significant extent in exponentially grown *E. coli*. The estimation of the upper limit of hexose resynthesis is about 3%.

In every case, the deoxyribosides contained a similar distribution of ^{18}O as the ribosides. This result was not unexpected, since the biosynthesis of deoxyribonucleotides is known to proceed through direct enzymatic reduction of ribonucleotides.

The results of the experiments described here leads to the conclusion that both the oxidative and nonoxidative pathways participate in the formation of pentose contained in the nucleic acids. Approximately 70% of the pentose is produced by

the nonoxidative pathway and 30% by the oxidative pathway. These values are in agreement with that of Model and Rittenberg (1967) who found that about 24% of the glucose consumed by exponentially growing *E. coli* is metabolized by way of the oxidative pathway. In that work, the specific yield of ^{18}O in CO_2 produced from [1-^{18}O]glucose was measured.

Recent investigations (Rutter, 1964) of the enzyme aldolase from several sources, including *E. coli*, indicates that the bacterial enzyme is different from the muscle enzyme in both its physical properties and mechanism of action. Unlike muscle aldolase, the bacterial enzyme shows an absolute requirement for a divalent metal ion. Its activity is not significantly inhibited after treatment with $NaBH_4$ and fructose diphosphate, but is completely lost on treatment with EDTA. Carboxypeptidase produces 100% inhibition of the activity of bacterial aldolase, while producing only 8% inhibition of the muscle enzyme.

Muscle aldolase has been shown to form a Schiff base between a lysine residue and the C-2 carbon atom of fructose diphosphate (Horecker *et al.*, 1963). The formation of this linkage necessitates the loss of the C-2 oxygen atom of the hexose to the medium. Model *et al.* (1968) have investigated the oxygen-exchange reaction of ^{18}O-labeled fructose phosphates with water, catalyzed by rabbit muscle aldolase. Their results are consistent with a Schiff base mechanism.

In the experiments described in this paper, *E. coli* grown on [2-^{18}O]glucose and [2-^{18}O]fructose produced a nucleoside containing a relatively large concentration of label in the pentose moiety. Consideration of the pathways for pentose biosynthesis lead to the conclusion that the ^{18}O in the 4′-oxygen atom of the pentose arose from [2-^{18}O]triose phosphate. Therefore, the aldolase cleavage of the [2-^{18}O]hexose did not lead to the loss of the ^{18}O label. In addition, this shows that the half-life for the exchange of dihydroxyacetone phosphate is *greater* than the lifetime of the molecule in the cell. These observations are compatible with the idea that *E. coli* contains an aldolase whose mechanism of action involves a metal chelate intermediate and not a Schiff base.

Acknowledgments

The authors are indebted to Dr. L. Ponticorvo for her help in the preparation of some of the ^{18}O-labeled compounds and also to Mr. J. Carter Cook, whose expert care of the mass spectrometers allowed accurate analyses to be obtained routinely.

References

Beynon, J. H. (1960), Mass Spectrometry and Its Applications to Organic Chemistry, Amsterdam, Elsevier, p 294.

Biemann, K., and McCloskey, J. A. (1962), *J. Am. Chem. Soc. 84*, 2005.

Caprioli, R., and Rittenberg, D. (1968a), *Proc. Natl. Acad. Sci. U. S. 60*, 1379.

Caprioli, R., and Rittenberg, D. (1968b), *Proc. Natl. Acad. Sci. U. S. 61*, 1422.

Dekker, C. J. (1965), *J. Am. Chem. Soc. 87*, 4027.

Dirheimer, G., and Ebel, J. P. (1967), *Bull. Soc. Chem. Biol. 49*, 447.

Dixon, M., and Webb, E. (1964), Enzymes, 2nd ed, New York, N. Y., Academic, p 278.

Entner, N., and Doudoroff, M. (1952), *J. Biol. Chem. 196*, 853.

Fraenkel, D. G., Falcoz-Kelly, F., and Horecker, B. L. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 1207.

Fraenkel, D. G., and Levisohn, S. R. (1967), *J. Bacteriol. 93*, 1571.

Hanessian, S., De Jongh, D., and McCloskey, J. A. (1966), *Biochim. Biophys. Acta 117*, 480.

Hardegger, E., and Montavon, R. M. (1946), *Helv. Chim. Acta 29*, 1199.

Hassan, M. W., and Lehninger, A. L. (1956), *J. Biol. Chem. 223*, 123.

Hiatt, H. H., and Lareau, J. (1958), *J. Biol. Chem. 233*, 1024.

Honeyman, J. (1963), *Methods Carbohydrate Chem. 2*, 95.

Horecker, B. L., Rowley, P. T., Grazi, E., Cheng, T., and Tchola, O. (1963), *Biochem. Z. 338*, 36.

Horecker, B. L., and Wood, W. A. (1957), *Methods Enzymol. 3*, 152.

Koshland, D. E., and Springhorn, S. S. (1956), *J. Biol. Chem. 162*, 421.

Lowry, O., and Lopez, J. (1946), *J. Biol. Chem. 162*, 421.

McCloskey, J. A., Lawson, A. M., Tsuboyama, K., Freuger, P. M., and Stillwell, R. N. (1968), *J. Am. Chem. Soc. 90*, 4182.

Model, P., Ponticorvo, L., and Rittenberg, D. (1968), *Biochemistry 7*, 1339.

Model, P., and Rittenberg, D. (1967), *Biochemistry 6*, 69.

Nicholson, J. F., Ponticorvo, L., and Rittenberg, D. (1965), *Biochim. Biophys. Acta 108*, 519.

Rieder, S., and Rose, I. (1959), *J. Biol. Chem. 234*, 1007.

Rittenberg, D., and Graff, C. (1958), *J. Am. Chem. Soc. 80*, 3370.

Rittenberg, D., and Ponticorvo, L. (1956), *J. Appl. Rad. Isotopes 1*, 208.

Rittenberg, D., and Ponticorvo, L. (1962), *J. Biol. Chem. 237*, PC2709.

Rittenberg, D., Ponticorvo, L., and Borek, E. (1961), *J. Biol. Chem. 236*, 1769.

Roberts, R. B., Cowie, D. B., Abelson, P. H., Botten, E. T., and Britten, R. J. (1957), Studies of Biosynthesis of *Escherichia coli*, Washington, D. C., Carnegie Institute, p 5.

Robison, R., and King, E. J. (1931), *Biochem. J. 25*, 323.

Rutter, W. J. (1964), *Federation Proc. 23*, 1248.

Sable, H. Z., and Cassisi, E. E. (1962), *J. Bacteriol. 84*, 1169.

Szynkiewicz, A., Sable, H., and Pflueger, E. (1961), *J. Bacteriol. 81*, 837.

Todd, A. R. (1957), *Methods Enzymol. 3*, 819.

White, L. M., and Secor, G. E. (1953), *J. Am. Chem. Soc. 75*, 6343.

Zablotny, R., and Fraenkel, D. G. (1967), *J. Bacteriol. 93*, 1579.