The Labeling of Pentose Phosphate from Glucose-^{14}C and Estimation of the Rates of Transaldolase, Transketolase, the Contribution of the Pentose Cycle, and Ribose Phosphate Synthesis*

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ABSTRACT: Labeling in pentoses formed from glucose-1-^{14}C and glucose-2-^{14}C has been used by several authors to determine the relative amounts of pentose which are formed by the oxidative and nonoxidative branches of the pentose pathway. These calculations have been interpreted to indicate that pentose is formed from hexose *via* the nonoxidative as well as *via* the oxidative branch of this pathway. It is shown here that these calculations often are misleading since they do not necessarily measure the net flow of carbon; instead they frequently, in large part, measure the incorporation of ^{14}C through exchange catalyzed by the reversible transaldolase and transketolase reactions. Equations have been derived using the distribution of ^{14}C in the carbons of hexose phosphate and pentose phosphate derivatives formed from glucose-1-^{14}C or glucose-2-^{14}C which permit calculation of the net flow of carbon by the pentose cycle and also the rates of the transketolase and transaldolase reactions. Using published data obtained with *Escherichia coli*, regenerating rat liver *in vivo*, and rat muscle and Hela cells *in vivo*, it has been shown that in all cases the net flow of carbon in the nonoxidative branch is from pentose to hexose and in the oxidative branch from hexose to pentose. Thus the net synthesis of pentose from hexose is caused entirely by the oxidative branch of the pentose cycle even though its rate is less than that of the reversible rates of the transketolase and transaldolase reactions. The rate of synthesis of phosphoribosyl pyrophosphate and its derivatives (nucleotides and nucleic acids) was evaluated using published data from *E. coli* and mammalian tissue. This synthesis accounts for only a small fraction of the pentose-5-P formed by decarboxylation of glucose-6-P. The effect of nonequilibration of the pentose-5-P esters (ribulose-5-P, xylulose-5-P, and ribose-5-P) on the labeling patterns of the hexose and pentose phosphate esters was examined. It is concluded that incomplete equilibration of the ^{14}C of the pentoses by epimerase and isomerase has much less effect on the labeling patterns of the hexose phosphates than it does on that of the pentose phosphates or sedoheptulose phosphate. Previous methods derived for estimation of the contribution of the pentose cycle and Embden–Meyerhof pathway to glucose metabolism also have not provided for the changes in ^{14}C patterns which would be caused by exchange reactions due to the reversible transketolase and transaldolase reactions. It has been found that the exchange reactions likewise influence the ratios of ^{14}C in carbons of the pentose phosphates much more than they do those of the hexose phosphates. Therefore, even though it was assumed that the transketolase and transaldolase reactions were unidirectional and that the ^{14}C of the three pentoses was equilibrated, previous methods for the estimation of the pentose cycle were reasonably accurate. It has been shown that the ^{14}C patterns in sedoheptulose would be quite useful for calculation of the pathways.

The pathways of synthesis of the ribose moiety of nucleotides and nucleic acids have been extensively investigated in the past 10 years (for reviews, see Horecker, 1962, 1965; Hollman, 1964; Potter, 1960). It is commonly held that in many organisms pentose-P[1] is formed from hexose-6-P by two mechanisms frequently designated as the "oxidative" and "nonoxidative" pentose phosphate pathway. The oxidative path proceeds *via* oxidation to phosphogluconate and its decarboxylation to pentose-5-P. The second path is

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[1] Abbreviations: TK, transketolase; TA, transaldolase; G-6-P, glucose-6-P; F-6-P, fructose-6-P; H-6-P, hexose-6-P; P-5-P, pentose-5-P; S-7-P, sedoheptulose-7-P; E-4-P, erythrose-4-P; GAP, glyceraldehyde-3-P; DHAP, dihydroxyacetone-P; Tr-3-P, triose-3-P; Ru-5-P, ribulose-5-P; Ri-5-P, ribose-5-P; Xu-5-P, xylulose-5-P; PRPP, phosphoribose pyrophosphate; PC, pentose cycle; EM path, Embden–Meyerhof pathway; NTP pathways, nontriose-P pathways, *i.e.*, pathways not involving triose-P formation.

catalyzed by the enzymes transketolase (TK) and transaldolase (TA), according to the over-all reaction

$$2\text{fructose-6-P} + \text{glyceraldehyde-3-P} \rightleftharpoons 3\text{pentose-5-P} \quad \text{(A)}$$

These views are based on studies in which glucose-1-^{14}C and glucose-2-^{14}C were administered and the labeling patterns were examined in pentose from RNA or DNA. It was reasoned that if the pathway for pentose synthesis was *via* decarboxylation of hexose-6-P, glucose-1-^{14}C would yield unlabeled ribose, and glucose-2-^{14}C would give a ribose labeled in carbon 1. It was found that in several mammalian tissues and microorganisms the pentose derived from glucose-1-^{14}C was extensively labeled in carbon 1. It was concluded that this labeling pattern reflected synthesis by the nonoxidative pathway. On the other hand, Wood *et al.* (1963) suggested that the labeling pattern in pentose-P resulted from ^{14}C exchange owing to the reversibility of the transketolase and transaldolase reactions and was not necessarily related to the synthesis of pentoses.[2]

Previous studies of ^{14}C randomization from glucose have not attempted to analyze quantitatively the combined effects of reversible transketolase and transaldolase. The purpose of the present paper is to present an examination of labeling patterns in pentose-5-P and other intermediates of the pentose cycle as affected by these reactions. Such analysis elucidates the contribution of pathways in the synthesis of the pentose of RNA and DNA. It also permits estimation of the steady-state rates of reversible reactions, including transaldolase and transketolase, in intact tissues.

Theory

Reversible and Irreversible Reactions. In analyzing the labeling pattern from glucose-^{14}C, it is necessary to distinguish between three types of reaction. These are: (I) irreversible reactions, (II) reversible reactions, and (III) rapidly reversible reactions in which the reactants are at complete isotopic equilibrium. Irreversible reactions are the phosphorylation of glucose and fructose-6-P, the decarboxylation of glucose-6-P to ribulose-5-P (by virtue of hydrolysis by lactonase), the synthesis of hexose-6-P derivatives *via* uridine diphosphoglucose (UDPG), and synthesis of pentose-5-P derivatives *via* phosphoribosyl pyrophosphate (PRPP).

On the other hand, reactions catalyzed by phosphohexose isomerase, triose-P isomerase, pentose-5-P isomerase and epimerase, aldolase, transaldolase, and transketolase are reversible. In the simplified model of glucose metabolism of Katz and Wood (1960), the rates of isomerization and epimerization were assumed to be very rapid so that the respective hexose 6-phosphates, triose phosphates, and pentose 5-phosphates were isotopically equilibrated. Thus within each of these three pools the phosphate esters are of equal specific activity and have an identical distribution of ^{14}C. In subsequent papers the number of restrictions was reduced and it was not assumed that the hexose phosphates (Landau *et al.*, 1964) and triose phosphates (Katz *et al.*, 1966) were at isotopic equilibrium. Methods were derived to estimate the steady-state rates of hexose-6-P and triose-P isomerase reactions when their rates were not sufficiently rapid to equilibrate the ^{14}C in the phosphate esters (Wood *et al.*, 1963; Landau *et al.*, 1964; Katz *et al.*, 1966). In adipose tissue the rates of isomerization of hexose-6-P and triose-P are quite high and equilibration is extensive. It is likely that this applies to many other tissues.

In previous models also transketolase and transaldolase were assumed to be irreversible and unidirectional, *i.e.*, to operate only in the direction of transformation of pentose phosphates to fructose 6-phosphates. A more general model without these restrictions will be considered in this paper, and methods to estimate the rates of these two reactions will be derived.

Model

Figure 1 presents a simplified model, showing glucose metabolism *via* the Embden–Meyerhof pathway, the pentose cycle, and synthesis of PRPP and hexose-6-P derivatives (UDPG). The reactions catalyzed by transketolase and transaldolase are reversible. Rates of isomerization and epimerization are considered very rapid and thus the hexose 6-phosphates, triose phosphates, and pentose 5-phosphates are isotopically equilibrated and each group of esters constitutes a single pool. Subsequently a more general model without these

[2] Conventions used by us (Wood *et al.*, 1963) are illustrated by the following example. Assume that 10 μmoles of glucose-6-P was converted to 10 μmoles of pentose-5-P and 10 μmoles of CO_2 per unit time by the oxidative pentose phosphate pathway while 100 μmoles of pentose-5-P was formed from fructose-6-P by the nonoxidative pathway. Of these 110 μmoles of pentose-5-P assume 1 μmole was converted to pentose of nucleic acid/unit time while 109 μmoles was converted to fructose-6-P through reversal of the nonoxidative pathway. The *net* flow of carbon in the nonoxidative pathway clearly would be from pentose-5-P to fructose-6-P and the *net* supply of carbon for the synthesis of the pentose of nucleic acid would be from the oxidative pathway and not the nonoxidative pathway. With glucose-^{14}C the ^{14}C pattern of the pentose would be in the above example influenced more by the nonoxidative pathway than by the oxidative pathway, but this would not indicate the direction of net flow of carbon. Of the 10 μmoles of pentose-5-P formed by the oxidative pathway 9 μmoles would complete the cycle by the nonoxidative branch (reaction A, right to left) and would yield 6 μmoles of fructose-6-P and 3 μmoles of glyceraldehyde-6-P. Therefore of the 10 μmoles of glucose-6-P formed in the oxidative pathway, 6 μmoles is regenerated, 1 μmole is converted to pentose of nucleic acid and 1 μmole of CO_2, and 3 μmoles of glucose-6-P is metabolized by the pentose cycle yielding 9 μmoles of CO_2 and 3 μmoles of glyceraldehyde-3-P. Thus if V_1 is the rate of decarboxylation of glucose-6-P and V_R is the rate of formation of PRPP, then $(V_1 - V_R)/3$ equals the rate of metabolism *via* the pentose cycle (see Figure 1). The regenerated 6 μmoles of fructose-6-P does not represent *net* metabolism of glucose. The 1 μmole of pentose-5-P which is converted to nucleic acid–pentose was not considered by our previous definition (Wood *et al.*, 1963) as metabolism by the pentose cycle but was included under nontriose-P pathways. This pathway also includes such reactions as the conversion of glucose-6-P to glycogen.

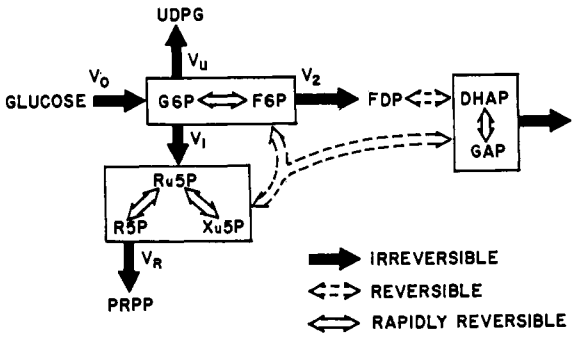

FIGURE 1: Model of glucose metabolism. Solid arrows, irreversible reactions; hollow arrows, very rapid reversible reactions; compounds in boxes in isotopic equilibrium. Broken arrows, reversible reactions catalyzed by transaldolase and transketolase. For abbreviations, see footnote 1.

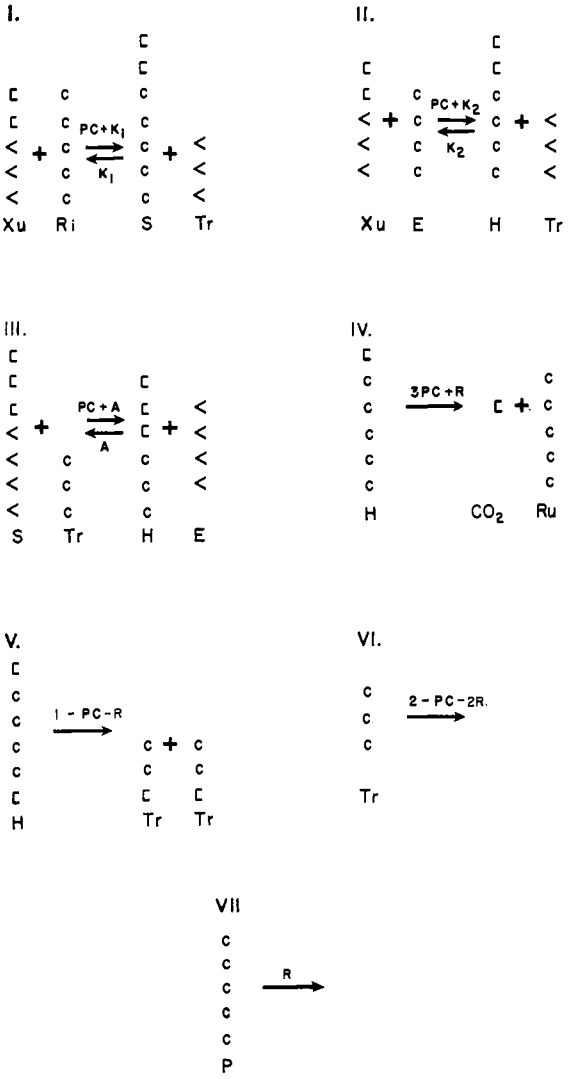

FIGURE 2: Carbon randomization in the operation of the pentose cycle and the Embden–Meyerhof pathway. Expressions I and II, transketolase; expression III, transaldolase; expression IV, decarboxylation of glucose-6-P to ribulose-5-P; expression V, cleavage of fructose-diP to two triose-P; expression VI, outflow from triose-P into glycerol, pyruvate, etc.; and expression VII, outflow from pentose-5-P into phosphoribosyl pyrophosphate.

latter restrictions will be considered.

In Figure 1 the rate of glucose phosphorylation is designated by V_0, the decarboxylation of glucose-6-P to ribulose-5-P by V_1, and the formation of fructose-1,6-diP by V_2. The rate of synthesis of PRPP is designated by V_R and that of UDP-glucose by V_u. Fructose-1,6-diP is cleaved to triose-P, which flows out of the system into phosphoglycerol and pyruvate and their derivatives. Rates are expressed in moles per unit time.[3] The transfer of carbon in the individual reactions is depicted in Figure 2. It follows from Figures 1 and 2 that if V_1 is greater than V_R (that is, formation of ribose-5-P by decarboxylation exceeds its outflow into PRPP), $(V_1 - V_R)$ moles of pentose-5-P is reconverted (recycled) by reactions catalyzed by transketolase and transaldolase to $^2/_3(V_1 - V_R)$ moles of fructose-6-P (reaction A, right to left). Likewise $(V_1 - V_R)/3$ moles of glucose is converted to glyceraldehyde-3-P (GAP). In this case, the synthesis of pentose-5-P derivatives proceeds solely *via* the "oxidative" path (see footnote 2).

If V_R is greater than V_1, then $(V_R - V_1)$ moles of pentose-5-P is formed from fructose-6-P and glyceraldehyde-3-P *via* reactions catalyzed by transketolase and transaldolase (reaction A). In this case, synthesis of pentose-5-P derivatives would proceed by both oxidative and nonoxidative pathways. There is then no recycling of pentose-5-P and no pentose cycle ($PC = 0$).

Derivation of Equations

Equations When the Net Flow of Carbon Is to Pentose-P via the Oxidative Pathway. This situation requires that V_1 exceed V_R. The condition to be considered is with no synthesis of UDPG from hexose-6-P derivatives (no nontriose-P pathways, $V_u = 0$). Other conditions will be dealt with subsequently. For convenience the rates are expressed as molar relative rates by dividing all rates by V_0, the rate of glucose utilization. The fractional rate of PRPP synthesis (V_R/V_0) is designated by R. Likewise, following our previous convention (Wood *et al.*, 1963) the fractional rate of decarboxylation of glucose-6-P (V_1/V_0) is equal to $3PC + R$ and the fractional rate of glucose metabolism *via* the Embden–Meyerhof pathway (V_2/V_0) is then $1 - PC - R$. The formation of triose-P and outflow to glycerol

[3] In previous papers (Wood *et al.*, 1963; Katz and Wood, 1960; Landau *et al.*, 1964) rates were expressed on the basis of glucose as *microatoms* of *carbon* per unit time. The choice of molar rates in the present paper is based on mathematical convenience in the derivations. The two units of rate are of course readily interconvertible.

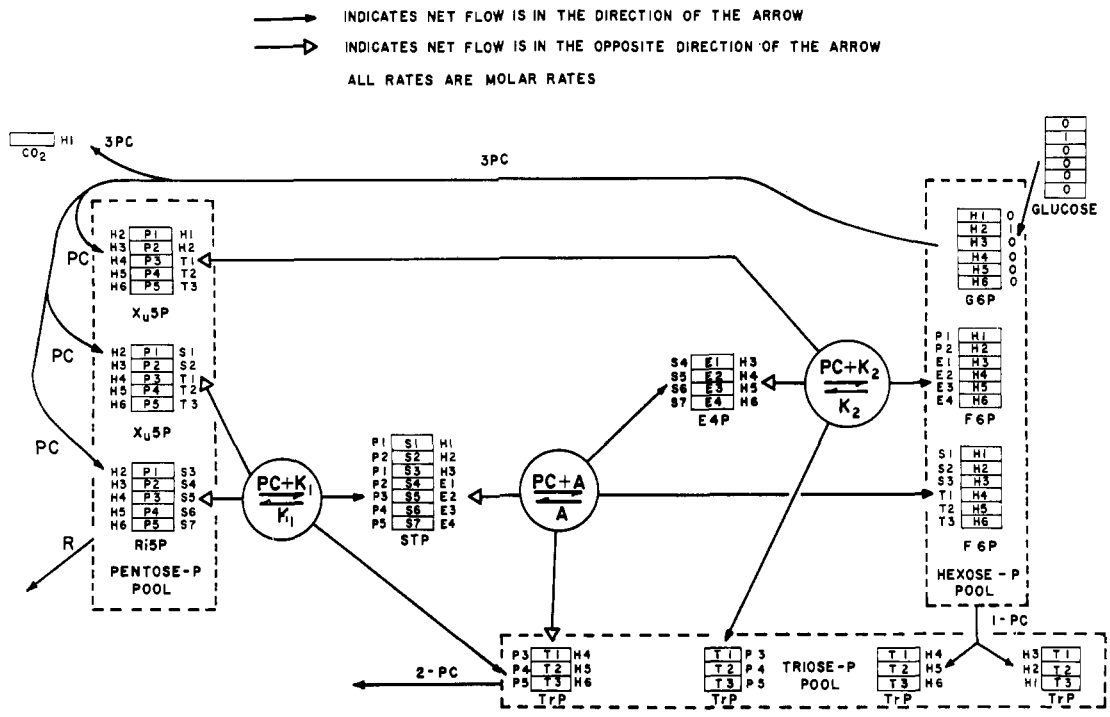

FIGURE 3: Integrated model showing carbon flow into all positions of compounds involved in the pentose cycle. The substrate is glucose-2-^{14}C. Letters outside the boxed compounds represent the source of the carbon flowing into each position by the reactions shown by the arrows. Compounds in boxes are isotopically equilibrated.

and pyruvate derivatives is $2(1 - PC - R) + PC = (2 - PC - 2R)$ moles/mole of utilized glucose.

The transformation of pentose-5-P and fructose-6-P occurs *via* two reversible reactions catalyzed by transketolase (expressions I and II, Figure 2) and one reversible reaction catalyzed by transaldolase (expression III, Figure 2). When V_1 exceeds V_R, the *net* flow of carbon is from pentose-5-P to fructose-6-P and triose-P, from left to right in expressions I–III of Figure 2. In the pentose cycle 3 moles of glucose-6-P is turned over/unit time and 2 moles of glucose-6-P is regenerated. In this process three pentose phosphates have turned over, one in the transaldolase reaction and two in the transketolase reactions. Thus the *net* rate of each of the three reactions (I–III of Figure 2) from left to right is equal to PC. Since the reactions are reversible the actual rates in either direction may be much greater. The actual rates from right to left is indicated as K_1, K_2, and A and therefore from left to right they are $PC + K_1$, $PC + K_2$, and $PC + A$ as shown in Figure 2 (I–III). If the rates of the reactions catalyzed by transketolase are assumed to be equal ($K_1 = K_2$), the subscript will be omitted.[4]

In expressions I–III two types of randomization occur: (1) one that randomizes the "top" carbons of the phosphate esters (H_1, H_2, H_3, P_1, P_2, S_1–S_4, and E_1);[5] and (2) one that exchanges the three "bottom" carbons of these esters in the transaldolase reaction. If both TK and TA are reversible, extensive rearrangement of the "top" carbons occurs.

Derivation of Equations for the Randomization of ^{14}C into the "Top" Carbons of Phosphate Esters. An expanded model of Figure 1, which integrates the individual reactions of Figure 2, is presented in Figure 3. In this figure the flow of carbon into each position is shown. It is apparent that (in the absence of outflow from the hexose-6-P and pentose-5-P pools) the randomization of ^{14}C from either glucose-1-^{14}C, -2-^{14}C, or -3-^{14}C is a function of four independent variables, PC, K_1, K_2, and A. If outflow from the pentose-5-P pool occurs, a fifth variable R must also be included. Procedures designed to evaluate the labeling pattern

[4] An example illustrates the numerical relationship between the terms K and A and the rates of transketolase and transaldolase. For example, let us assume that $PC = 0.1$ (10% pentose cycle contribution), $K = 0.5$, $A = 0.7$, and the rate of glucose utilization is 50 μmoles/hr. The rate of glucose-6-P oxidation to phosphogluconate (or formation of ribulose-5-P) is $3 \times 0.1 \times 50 = 15$ μmoles/hr. The total rate of transformation of xylulose-5-P by transketolase $(2 \times 0.1 + 2 \times 0.5)50 = 60$ μmoles/hr (of which one-half reacts in expression I and one-half reacts in expression II). The cleavage of sedoheptulose-7-P by transaldolase is $(0.1 + 0.7)50 = 40$ μmoles/hr. In the reverse direction the two transketolase reactions are 25 μmoles/hr each, and transaldolase 35 μmoles/hr.

[5] The carbons of hexose-6-P (G-6-P + F-6-P) are represented by H. The carbons of the pentose (Xu-5-P + Ri-5-P + Ru-5-P) are represented by P. DHAP and GAP also constitute a single triose-P pool and the carbons are represented by Tr. The individual carbons of the hexose-6-P, pentose-5-P, sedoheptulose-7-P, erythrose-4-P, and triose-3-P are designated as H_1–H_6, P_1–P_5, S_1–S_7, E_1–E_4, and Tr_1–Tr_3 respectively.

SCHEME I

Ester	Position	Inflow		Outflow	Eq
Hexose-6-P	H_1	$1 + P_1(PC + K_2) + S_1(PC + A)$	=	$H_1(1 + 2PC + A + K_2)$	1
	H_2	$P_2(PC + K_2) + S_2(PC + A)$	=	$H_2(1 + 2PC + A + K_2)$	2
	H_3	$E_1(PC + K_2) + S_3(PC + A)$	=	$H_3(1 + 2PC + A + K_2)$	3
Pentose-5-P	P_1	$H_1K_2 + H_2(3PC + R) + (S_1 + S_3)K_1$	=	$P_1(3PC + 2K_1 + K_2 + R)$	4
	P_2	$H_3(3PC + R) + H_2K_2 + (S_2 + S_4)K_1$	=	$P_2(3PC + 2K_1 + K_2 + R)$	5
Sedoheptulose-7-P	S_1	$P_1(PC + K_1) + H_1A$	=	$S_1(PC + A + K_1)$	6
	S_2	$P_2(PC + K_1) + H_2A$	=	$S_2(PC + A + K_1)$	7
	S_3	$P_1(PC + K_1) + H_3A$	=	$S_3(PC + A + K_1)$	8
	S_4	$P_2(PC + K_1) + E_1A$	=	$S_4(PC + A + K_1)$	9
Erythrose-4-P	E_1	$S_4(PC + A) + H_3K_2$	=	$E_1(PC + A + K_2)$	10

SCHEME II

Ester	Position	Inflow		Outflow	Eq
Hexose-6-P	H_6	$E_4(PC + K_2) + Tr_3(PC + A)$	=	$H_6(1 + 2PC + A + K_2)$	11
Pentose-5-P	P_5	$S_7K_1 + Tr_3(K_1 + K_2) + H_6(3PC + R)$	=	$P_5(3PC + 2K_1 + K_2 + R)$	12
Sedoheptulose-7-P	S_7	$P_5(PC + K_1) + E_4A$	=	$S_7(PC + A + K_1)$	13
Erythrose-4-P	E_4	$S_7(PC + A) + H_6K_2$	=	$E_4(PC + A + K_2)$	14
Triose-P	Tr_3	$P_5(2PC + K_1 + K_2) + H_6(1 + A - PC - R) + H_1(1 - PC - R)$	=	$Tr_3(2 + K_1 + K_2 + A - 2R)$	15

have been previously developed (Wood *et al.*, 1963; Katz and Wood, 1960; Landau *et al.*, 1964). It is necessary to write equations for inflow and outflow of ^{14}C for each carbon. If the system is in metabolic and isotopic steady state (Wood *et al.*, 1963; Landau *et al.*, 1964), ^{14}C inflow equals ^{14}C outflow. Such equations may be derived most readily by inspection of the expressions of Figure 2 or those of Figure 3. A general scheme for the derivation of such equations is described in Appendix A. The steady-state expressions for the ten top carbons derived from glucose-1-^{14}C, are given in eq 1–10 of Scheme I.[5] The equations for glucose-2-^{14}C or -3-^{14}C are identical with those for glucose-1-^{14}C, except that the term "1" in the inflow appears in eq 2 or 3, respectively.

Derivation of Equations for the Labeling of the Bottom Carbons. In previous papers where the transketolase and transaldolase were assumed to be unidirectional (Wood *et al.*, 1963; Katz and Wood, 1960), it was pointed out that carbons 4–6 of glucose-6-P would become labeled by glucose-1-^{14}C or -2-^{14}C through pick up of glyceraldehyde-3-P by the operation of the pentose cycle. Dihydroxyacetone-P (DHAP), labeled from glucose-1-^{14}C or -2-^{14}C, equilibrates with GAP, which is reincorporated into hexose-6-P *via* the transaldolase reaction. Transaldolase exchange has been discussed by Landau and Bartsch (1966). Likewise, as shown in expressions I and II of Figure 2, by the reversible transketolase reaction, triose-P will exchange with carbons 3–5 of pentose. Thus by the combined effect of transketolase and transaldolase, ^{14}C is incorporated into the bottom carbons of all the esters.

In eq 11–15 of Scheme II, the steady-state equations of labeling of the bottom carbons H_6, P_5, S_7, E_4, and Tr_3 by glucose-1-^{14}C are shown.[6] When glucose-2-^{14}C is the labeled substrate, the major labeled bottom carbons would be H_5, P_4, S_6, Tr_2, and E_3 and, for glucose-3-^{14}C, H_4, P_3, etc. The equations are identical with those for glucose-1-^{14}C except that the numbering of the positions is changed, and the term H_1 in eq 15 is replaced with H_2 or H_3, respectively.

These 15 simultaneous linear equations can be solved algebraically for the 15 positions in terms of PC, K_1, K_2, A, and R; thus knowing the labeling in H_1, H_2, H_3, H_6, P_1, P_2, P_5, etc., one may determine PC, K_1, K_2, A, and R. The expressions are, however, of such length and complexity as to make solution by elementary procedures

[6] Carbons 4 and 5 of hexose-6-P, carbons 3 and 4 of pentose-P and the corresponding carbons in sedoheptulose-7-P and erythrose-4-P also became labeled. However, since $H_1:H_2:H_3 = H_6:H_5:H_4$, equations for the other two carbons in the "bottom" groups do not provide independent relationships, and the equations are not presented. This condition holds if glucose-6-P and fructose-6-P are isotopically equilibrated, as is assume here.

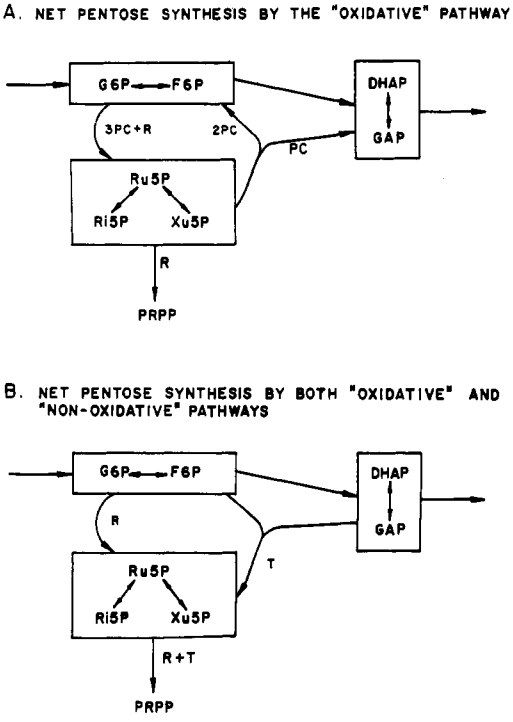

FIGURE 4: Net flow in the synthesis of pentose. (A) Net pentose synthesis by the oxidative pathway. Per mole of glucose, a fraction $3PC + R$ of glucose-6-P is decarboxylated, $2PC$ is recycled, and R converted to PRPP. (B) Net pentose synthesis by both oxidative and nonoxidative pathways. Per mole of glucose, a fraction R is formed by the oxidative and a fraction T by the nonoxidative path.

impractical. Numerical solution of the equations for any desired values of the parameters can be readily obtained by digital computer (see Appendix B).

When transketolase is unidirectional ($K_1 = K_2 = 0$), the equations simplify. The ratio H_6/H_1 can then be readily calculated from eq 11 to 15 and is presented (neglecting R) in eq 16.

$$H_6/H_1 = \frac{(1 - PC)(PC + A)}{(2 - PC)(1 + PC + A)} \qquad (16)$$

When A is 0, eq 16 reduces to eq 17.

$$H_6/H_1 = \frac{(1 - PC)PC}{(2 - PC)(1 + PC)} \qquad (17)$$

This represents the labeling of H_6 from H_1 only through the operation of the pentose cycle, if transketolase and transaldolase were unidirectional. Equations for labeling from glucose-2-^{14}C are analogous, since $H_5/H_2 = H_6/H_1$.

Equations Where There Is Outflow from the Hexose-6-P Pool into Nontriose-P Pathways or There Is Incomplete Equilibration of the Phosphate Esters of Hexose and Triose. In the model of Figure 3 synthesis of hexose-6-P derivatives (designated previously (Wood *et al.*, 1963; Katz and Wood, 1960) as nontriose-P pathways, NTP) such as glycogen or other polysaccharides was neglected. This outflow may be included, introducing a sixth parameter in eq 1–15. This parameter will appear only in the expression for outflow from the triose-P pool, in eq 15. This new parameter does not affect the labeling of the ten top carbons, but only of the bottom carbons. It may be shown that unless this outflow is large (above 10%) its effect is limited.

Equations 1–15 are based on the assumption of complete equilibration of glucose-6-P and fructose-6-P. When these esters are not in isotopic equilibrium, randomization in carbons 1–3 of fructose-6-P will exceed that of glucose-6-P (Wood *et al.*, 1963; Landau *et al.*, 1964; Landau and Bartsch, 1966). Equations 1–15 can be extended to include the rate of hexose-6-P isomerase, introducing an additional parameter. The four equations for hexose-6-P (eq 1–3 and 11) would be replaced by eight equations, four for glucose-6-P and four for fructose-6-P.

Another assumption of eq 1–15 is the complete equilibration of triose phosphates. Methods to evaluate this rate have been described (Katz *et al.*, 1966). As discussed elsewhere (Katz *et al.*, 1966), the assumption is not critical unless glycerol synthesis is extensive. If the rate of triose-P isomerase is included an additional parameter must be introduced, and eq 15 for triose-P replaced by two equations, one for GAP and one for DHAP. Aldolase is also a reversible reaction, but since no major outflow for synthesis from fructose-1,6-diP is known this reaction is not considered.

Equations When There Is Incomplete Equilibration of Pentose Phosphates. Another assumption underlying eq 1–15 is the complete equilibration of ribulose-5-P, xylulose-5-P, and ribose-5-P, catalyzed by an epimerase and an isomerase. The system of equations may be extended to include the effect of these two reactions (see Appendix A). Two new parameters are introduced, and eq 4, 5, and 12 for pentose-5-P will be replaced by nine equations, three each for ribulose-5-P, xylulose-5-P, and ribose-5-P, yielding a system of 21 simultaneous equations (see Appendix A).

Models of even greater complexity may be considered, and equations for such models written. A model including all the rates mentioned above would constitute a set of about 30 simultaneous equations with some ten parameters. While such a system may be analyzed by the use of computer techniques, experimental study of such a complex model provides formidable difficulties and in practice simplifying assumptions are necessary.

Synthesis of Phosphoribose Pyrophosphate (PRPP). In Figure 4 two models for the synthesis of PRPP and ultimately of the pentose of nucleic acids and nucleotides are shown. In Figure 4A, the fractional rate of ribulose-5-P formation through decarboxylation of glucose-6-P is designated by $3PC + R$. A part, R, is converted to PRPP, and another part, $3PC$, is converted (recycled) to fructose-6-P. While the TA- and TK-catalyzed reactions are reversible, the *net* flow of carbon is (Figure 4A)

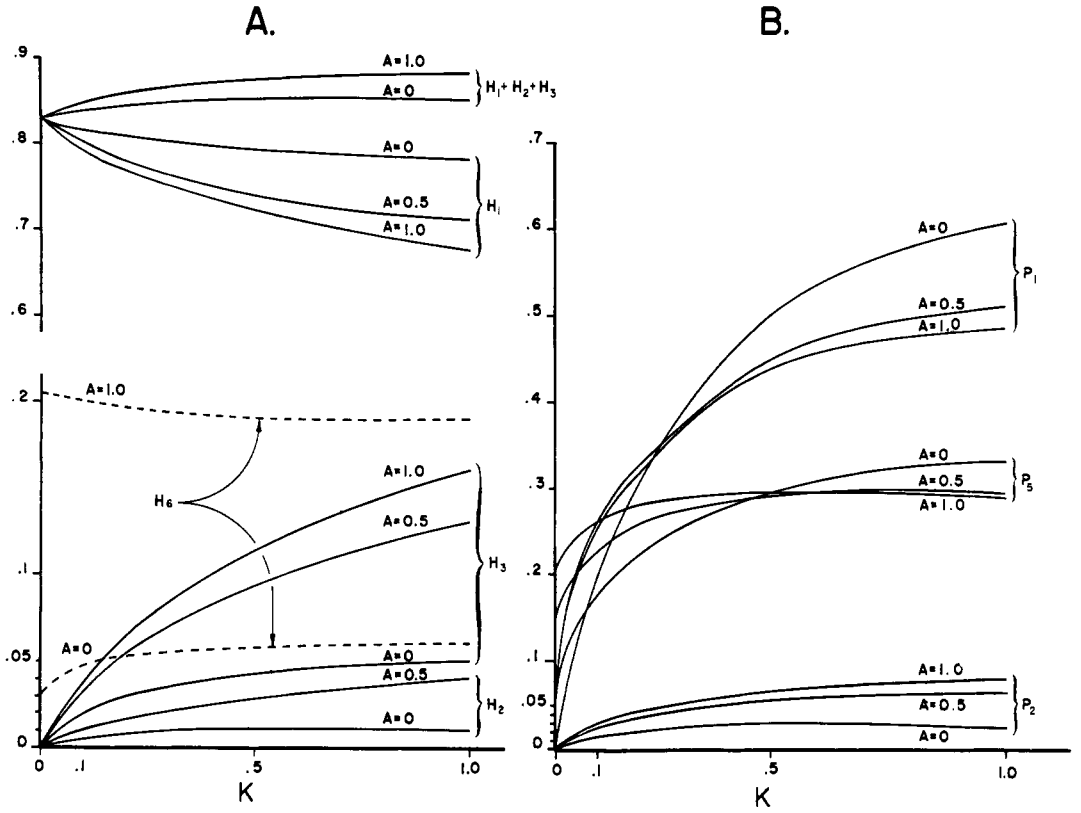

FIGURE 5: Relative specific activities of carbons of hexose-P and pentose-P from glucose-1-^{14}C as a function of K and A. Specific activity of carbon 1 of glucose is taken as 1.0. Pentose cycle set equal to 10%. Families of curves for $A = 0$, 0.10, and 1.0. (A) Specific activity in hexose-6-P. (B) Specific activities in pentose-5-P.

from pentose-5-P to fructose-6-P. Thus *net* synthesis of PRPP is only by the oxidative path.

In Figure 4B synthesis of PRPP by two pathways is depicted. The fractional decarboxylation of glucose-6-P to ribulose-5-P is R (oxidative path), the fractional rate of pentose synthesis catalyzed by transketolase and transaldolase is T (nonoxidative path), and total synthesis of PRPP is $R + T$. For each of expressions I–III of Figure 2 (right to left), the net rate of pentose-5-P synthesis is $T/3$. The reversible rates for the two reactions catalyzed by transketolase (expressions I and II) are K_1 or K_2 from left to right and $K_1 + (T/3)$ or $K_2 + (T/3)$ from right to left. The rate of transaldolase from left to right is A, in the reverse direction, $A + (T/3)$. Note that here *net* flow is in the opposite direction of the scheme of Figures 2 and 3. The derivation of the equations is analogous to eq 1–15; the equations are presented in Appendix A.

Effect of Variation of PC, the Transketolase Reaction (K_1, K_2), and the Transaldolase Reaction (A) on the Labeling Patterns of the Phosphate Esters

The specific activities of the carbons of the phosphate esters are a function of five parameters in eq 1–15 (PC, K_1, K_2, A, and R). In theory, for the solution of these equations the experimental determination of specific activities of any five carbons is sufficient. Owing to endogenous dilution, five ratios of specific activities of carbons in the same compound, rather than the specific activities themselves, are required. In practice, with glucose-1-^{14}C or -2-^{14}C as substrate, the most readily determined ratios are H_1/H_2, H_3/H_2, and H_6/H_1 (or H_5/H_2) from hexose of glycogen, and P_2/P_1 and P_5/P_1 (or P_4/P_2) from ribose of nucleic acids. At first the effect of the parameters on the specific activities and the ratios will be analyzed under the simplifying assumptions when there is no synthesis of PRPP ($R = 0$) and the two transketolase reactions are equal ($K_1 = K_2$). (Also, in eq 1–15 the hexose phosphates, pentose phosphates, and triose phosphates are assumed to be equilibrated.) More complex conditions will be considered later. Equations 1–15 and the more complex models presented below were solved by means of digital computer (see Appendix B).

In Figure 5, the relative specific activities of carbons 1–3 and 6 of hexose-6-P and carbons 1, 2, and 5 of pentose-5-P, derived from glucose-1-^{14}C, are plotted. The specific activities represent values that would be attained in the absence of any endogenous dilutions. The pentose cycle contribution was kept constant (10%). Families of curves for three values of transaldolase (0, 0.5, and 1.0) are plotted as a function of the rate of transketolase. If transaldolase and transketolase were unidirectional ($A = K = 0$), there would be transfer of ^{14}C from C-1 into C-6 of glucose-6-P (H_6), and into carbon 5 of pentose-5-P (P_5), but no label will be found in other posi-

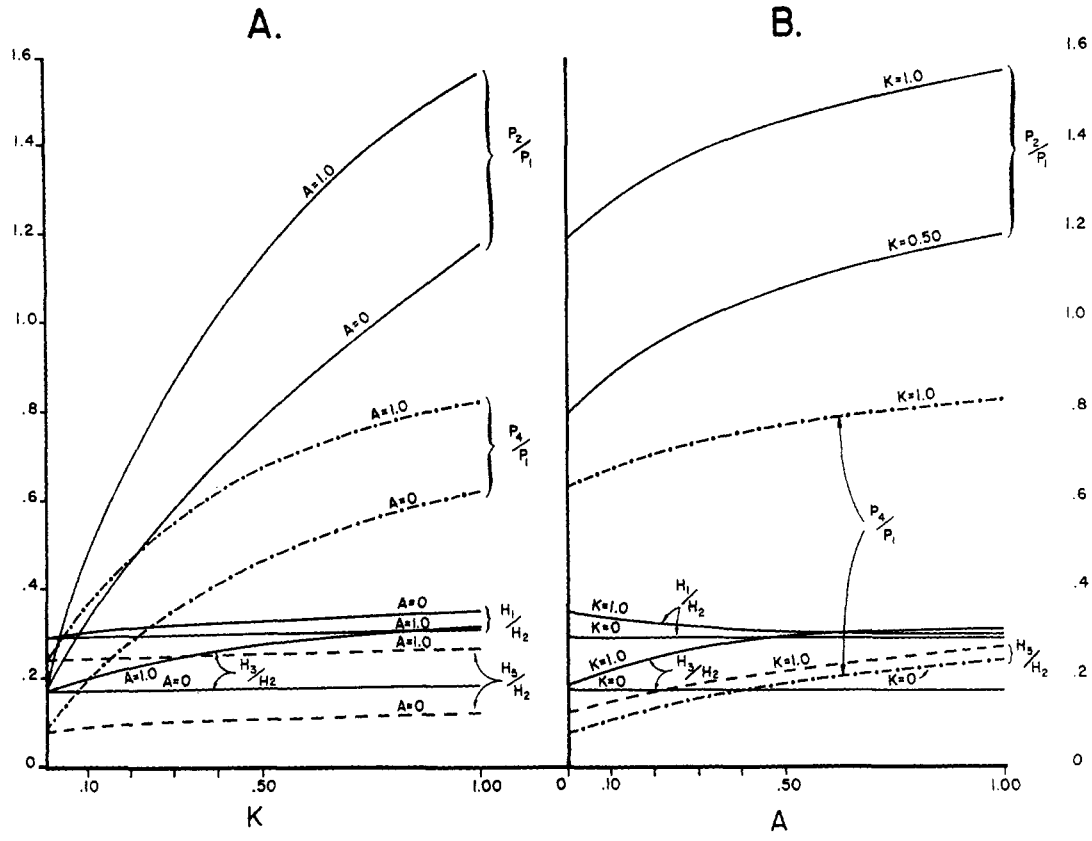

FIGURE 6: Ratios of specific activities in carbons of hexose-P and pentose-P from glucose-2-^{14}C. Pentose cycle set equal to 20%. (A) Transketolase is independent variable. (B) Transaldolase is independent variable.

tions. If transaldolase and transketolase function reversibly, label will appear in all positions. As these rates approach infinity, in the limit carbons 1–3 of hexose-6-P and carbons 1 and 2 of pentose-5-P would be equally labeled; the three bottom carbons of both esters would each be equally labeled and contain one-half the ^{14}C present in the top carbons.

Labeling of hexose is shown in Figure 5A, of pentose in Figure 5B. It is apparent that when *A* and *K* increase, the specific activity of H_1 slowly decreases. The ^{14}C in H_3 and H_2 increases but the activity of H_3 increases much faster than that in H_2. The ^{14}C in H_6 increases with *A*, but it is only slightly affected by *K*. The total ^{14}C label in the three top carbons of hexose-6-P ($H_1 + H_2 + H_3$), which represents the specific activity of triose-P, is barely affected by changes of *A* and *K*, since the gain of ^{14}C in H_3 and H_2 is compensated mostly by ^{14}C loss from H_1.[7]

The most striking aspect of Figure 5B is the very extensive incorporation of ^{14}C into carbon 1 of pentose-5-P (P_1). The extent of P_2 labeling is much less than that of P_1. With increase in transketolase, the activity in P_5 increases and surpasses that of H_6. The extent of labeling of P_1 and P_5 depends primarily on *K*, and is not much affected by A. Other carbons (H_4, H_5, P_3, and P_4) also became labeled, but the activity is low and not shown.

In Figure 6, the ratios of specific activities in hexose-6-P and pentose-5-P from glucose-2-^{14}C are presented. The pentose cycle contribution was kept constant at 20%. In Figure 6A *K* is the independent variable, and in Figure 6B it is *A*. It is apparent that in the hexose-6-P: (1) the ratio H_1/H_2 changes little with increases in *A* and *K;* (2) the ratio H_3/H_2 increases moderately with increase in *K* and *A;* (3) the ratio H_5/H_2 increases with *A*, but is very little affected by *K*.

The most pronounced effects are in the ratios in pentose-5-P. Carbon 2 of pentose-5-P (P_2) becomes very rapidly labeled, and when *K* and *A* are 0.5 becomes equal to P_1. The ^{14}C in P_4 also increases quite rapidly, depending primarily on *K* rather than *A*.

In Figure 7 the ratios of ^{14}C in the four top carbons of sedoheptulose-7-P from glucose-2-^{14}C are shown. It is apparent that the labeling pattern in sedoheptulose changes rapidly with the increase in *K* or *A*. It is especially sensitive to changes in *A*.

[7] In a previous paper (Katz, *et al.*, 1966), the difference in the ratios from glucose-1-^{14}C and -6-^{14}C in glycerol and fatty acids was attributed to incomplete equilibration of triose phosphates. However *randomization of* ^{14}C *from* glucose-1-^{14}C into C-3 of hexose-6-P may also contribute to the difference. It is apparent from Figure 5A that when *K* and *A* are appreciable, the specific activity of $C_1 + C_2$ from glucose-1-^{14}C will be less than that from $C_1 + C_2 + C_3$, and the C_1/C_6 ratio in lactate may exceed that in fatty acids.

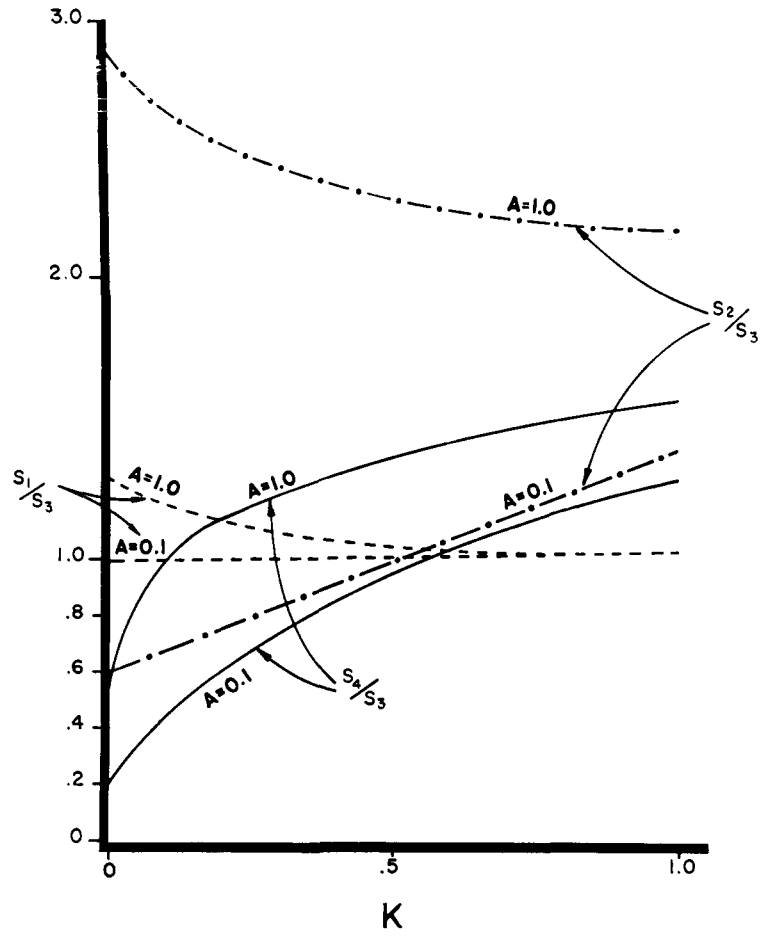

FIGURE 7: Ratios of specific activities in carbons of sedoheptulose-P from glucose-2-^{14}C as a function of K and A. Pentose cycle set equal to 20%. Families of curves for $A = 0.1$ and 1 and K varied from 0 to 1.0.

Distribution of ^{14}C When Synthesis of PRPP Is via the Oxidative Path. In Figure 8 the effects of outflow from the pentose-5-P pool (R) on the labeling pattern are shown. The contribution of the pentose cycle (10%) and K and A were kept constant ($K = A = 0.5$), and R varied from 0 to 0.3. At 0.3, R equals $3PC$ (3×0.1), one-half of the pentose-5-P formed by decarboxylation of glucose-6-P is recycled, and one-half is converted to PRPP. It is apparent from Figure 8 that outflow from the pentose-P pool (R) has little effect on ^{14}C in hexose-6-P. The most pronounced effect is a decrease in the ratios P_2/P_1 and P_4/P_1 in pentose-5-P from glucose-2-^{14}C, which is appreciable for values of R above 0.05. It was found that if the outflow, R, is less than 20% of the contribution of the pentose cycle (PC) the change in ratios is less than that due to experimental error and evaluation of R would not be practical from the ratios of ^{14}C in the phosphate esters.

More Complex Conditions. In the previous examples the two transketolase rates (K_1 and K_2 of Figure 2) were assumed to be equal. In Figure 9 the case when these two rates are unequal is illustrated. It is apparent that the ratios in hexose-6-P from glucose-2-^{14}C are not much affected by this condition, but the ratios in pentose, especially P_2/P_1, are quite sensitive. Unequal rates of the two transketolase reactions affect extensively the ^{14}C distribution in sedoheptulose.

Incomplete equilibration of the pentoses (ribulose-5-P, xylulose-5-P, and ribose-5-P) depends on two parameters (pentose-5-P epimerase and isomerase). The analysis is too lengthy to be presented in detail. The main conclusions are: (1) pentose epimerase and isomerase have little effect on the labeling of hexose-6-P; (2) the effect on the labeling pattern in two top carbons of pentoses and the four top carbons of sedoheptulose is pronounced; and (3) isotopic equilibration of the pentose phosphate is virtually complete when the rates of these reactions are 10–20 times that of the value of PC.

For example, when the pentose cycle is 20% and $K = A = 0.5$, and the rates of epimerase and isomerase are twice that of glucose utilization (ten times that of PC), the C_1/C_2 ratio in ribose and xylulose is about 80% that of equilibrium values. An experimental study of these rates would require isolation and degradation of the derivatives of the three pentoses or two pentose and sedoheptulose.

Distribution of ^{14}C When the Net Flow of Carbon to Pentose-P Is by both the Oxidative and Nonoxidative

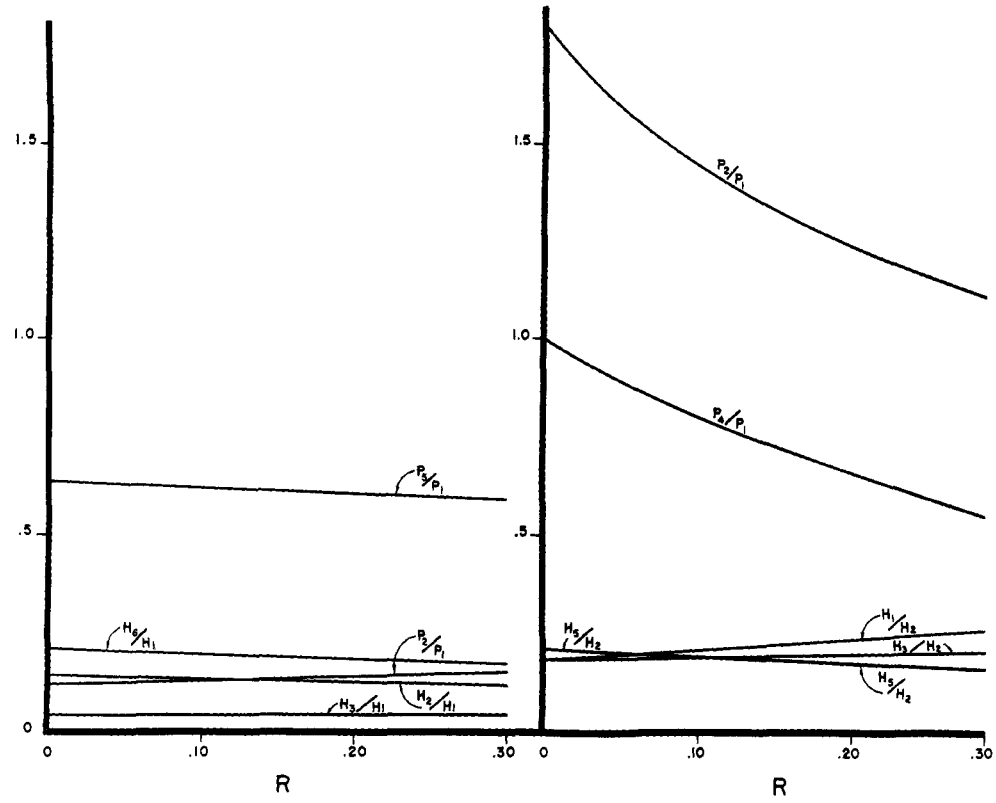

FIGURE 8: Effect of outflow from pentose-P pool (R) on ratios of specific activities in hexose-P and pentose-P. Pentose cycle set equal to 10%; A and K set equal to 0.50.

Pathways. In Figure 10 conditions are presented when the synthesis of pentose-P proceeds equally *via* the oxidative and nonoxidative pathways according to the model of Figure 4B. In this model there is no pentose cycle, *i.e.*, no net recycling of pentose-5-P to fructose-6-P. When transketolase and transaldolase are unidirectional in this system ($K = A = 0$), no randomization of ^{14}C in hexose-6-P occurs, and the pentose-5-P formed from glucose-2-^{14}C by decarboxylation is labeled only in carbon 1. The pentose-5-P formed *via* the nonoxidative path is labeled only in carbon 2, and the relative specific activity of this carbon is two-thirds that of C-2 of hexose-6-P. If both oxidative and nonoxidative pathways contribute equal amounts of pentose-5-P, the P_1/P_2 ratio is 1.5 (P_1/P_2 ratios are plotted in Figure 10 rather than the P_2/P_1 ratios used previously to avoid large numerical values). However, when transketolase proceeds reversibly, the P_1/P_2 ratio decreases precipitously. When K is 0.5 the P_1/P_2 ratio is 0.15. For the same rates of transketolase and transaldolase and when net formation of pentose-P occurs only by the oxidative pathway, this ratio is about 1. It is apparent that the ^{14}C distribution in hexose-6-P and pentose-5-P is much different for the two conditions (compare Figure 10 and Figure 6A).

To illustrate further the effects of net synthesis of pentose by both the oxidative and nonoxidative pathways, the distribution of ^{14}C has been calculated for all carbons of hexose-6-P and pentose-5-P from glucose-1-^{14}C and glucose-2-^{14}C for three conditions. The rates of transketolase and transaldolase were kept constant at 60 and 50 μmoles/unit time, respectively, expressed relative to glucose utilization which was 100 μmoles/unit time. The three conditions illustrated in Table I are: (1) 10% pentose cycle with no outflow from the pentose-5-P pool; (2) the same level of pentose cycle as in 1, with the additional synthesis of 10 μmoles of PRPP/unit time; and (3) no pentose cycle, but synthesis of 10 μmoles of PRPP per unit time, one-half net *via* the oxidative and one-half net *via* the nonoxidative path. As seen from Table I, the ^{14}C patterns for conditions 1 and 2 are similar. The only significant difference is the decrease in the P_2/P_1 ratio from glucose-2-^{14}C from 1.07 to 0.86. However, in condition 3 a very different pattern of ^{14}C distribution from glucose-2-^{14}C is revealed.

Calculation of PC, the Rates of the Transketolase (K) and Transaldolase (A) Reactions Using Published Data from E. coli and Mammalian Tissue

Estimation of the rates of transaldolase, transketolase, PRPP synthesis, or pentose-P equilibration requires the use of glucose-1-^{14}C, -2-^{14}C, or -3-^{14}C and isolation and degradation of phosphate esters or their derivatives. Specific activity ratios equal to the number of reaction rates to be determined are required. Several investigators have published results on the degradation of glucose

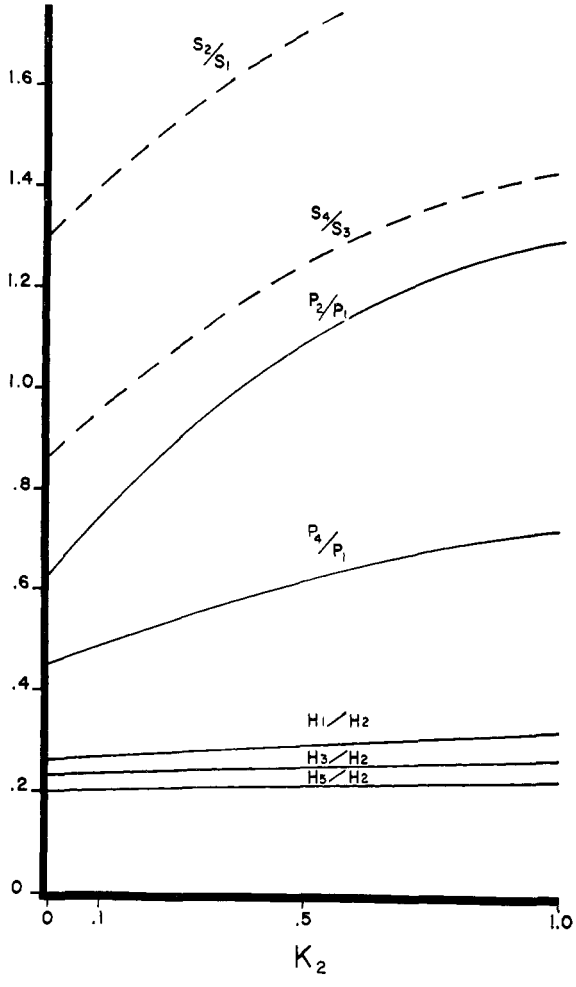

FIGURE 9: Ratios of specific activities in hexose-6-P and pentose-5-P from glucose-2-^{14}C, when the two rates catalyzed by transketolase are not equal. Pentose cycle contribution is 20%, $A = 0.5$, and $K_1 = 0.5$ (see Figure 2). K_2 is the independent variable.

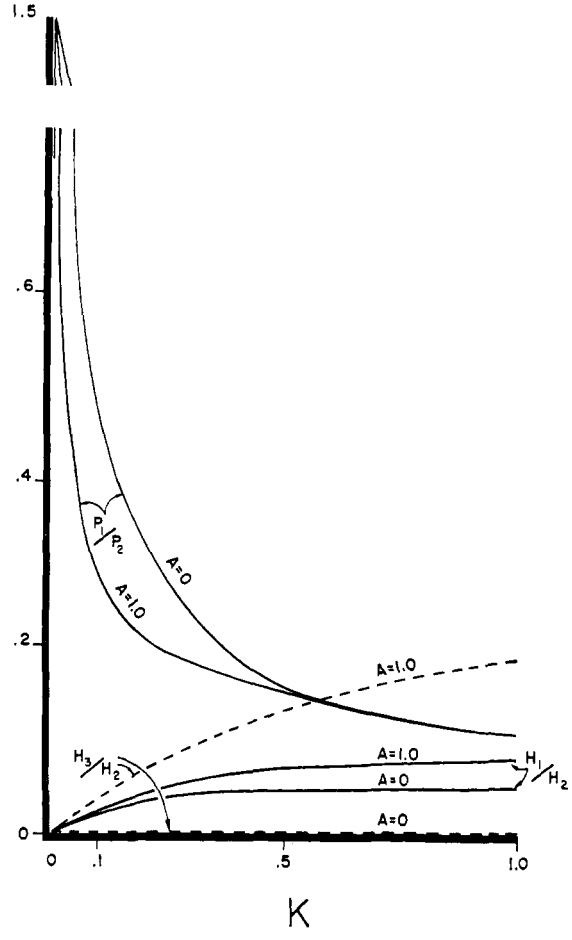

FIGURE 10: Ratios of specific activities in hexose-P and pentose-P from glucose-2-^{14}C when pentose-P synthesis proceeds to an equal extent from oxidative and nonoxidative pathways. $A = 0$ and 1.0, K varied from 0 to 1.0, $R = 0.06$, and $T = 0.06$.

of glycogen and pentose from RNA or DNA after incubation of organisms or tissues with labeled substrates. The degradation of a hexose and pentose provides five major ratios and theoretically five ratios should be adequate to evaluate five rates. If it is assumed that the hexose and pentose phosphates are completely equilibrated, the parameters might be PC, A, K_1, K_2, and R. If the rates of glucose utilization were known the rates of glucose-6-P dehydrogenase, transaldolase, and two transketolase reactions and the rate of PRPP synthesis could be obtained. However, the reliability of such an estimate would be very limited. This is due on one hand to the marked cumulative effects of experimental errors, and on the other hand to the large number of simplifying assumptions. To have reliable results the available ratios should exceed considerably the number of the parameters to be calculated. Numerous ratios may be obtained by simultaneous use of several labels, or by isolating several intermediates, including sedoheptulose, xylulose, etc.

In the analysis of the published data, we have assumed that the two rates of transketolase are equal and that the rate of PRPP synthesis is negligible. A justification for these assumptions will be presented subsequently. In addition it has been assumed that the isomerase and epimerase reactions are rapid, giving equilibration of ^{14}C in the hexose-P, pentose-P, and triose-P pools.

The procedure for the estimation of PC, K, and A based on the five ratios in glucose and pentose was as follows. (1) The pentose cycle was approximated from the H_1/H_2 ratio from glucose-2-^{14}C from the expression $PC = (H_1/H_2)/2(1 - (H_1/H_2))$ (Wood *et al.*, 1963; Katz and Wood, 1960). (2) The maximal value of A was obtained from the value of PC and the H_2/H_5 ratio in glucose by eq 17. (3) Using values of PC and A equal and less than these estimates, eq 1–15 were solved by a computer (see Appendix B) for a wide range of values of K. By trial and error a set of values for PC, A, and K were

TABLE I: Calculated Distribution of ^{14}C in Hexose-6-P and Pentose-5-P from Glucose-1-^{14}C and -2-^{14}C in the Presence of Reversible Transaldolase and Transketolase Reactions and the Effect of Synthesis of Phosphoribosyl Pyrophosphate

	Condition I		Condition II		Condition III	
Relative rates[a]						
Transketolase	60		60		60	
Transaldolase	50		50		50	
Glucose-6-P decarboxylation	30		40		5	
PRPP[b] synthesis	0		10		10	
Parameters						
PC	0.1		0.1		0	
A	0.4		0.4		0.5	
K	0.2		0.2		0.3	
R	0		0.1		0.05	
T					0.05	
	Hexose	Pentose	Hexose	Pentose	Hexose	Pentose
	Glucose-1-^{14}C					
Sp Act. of C-1[c]	0.78	0.35	0.76	0.30	0.87	0.61
Rel sp act. of carbons of hexose-P and pentose-P						
C-1	100	100	100	100	100	100
C-2	1.6	10	1.6	11	1.1	4.9
C-3	7.6	5.6	6.7	5.1	10	6.0
C-4	1.3	1.2	1.1	1.2	1.8	0.6
C-5	0.3	74	0.3	76	0.2	60
C-6	17		16		18	
	Hexose	Pentose	Hexose	Pentose	Hexose	Pentose
	Glucose-2-^{14}C					
Sp Act. of C-2 or C-1[c]	0.81	0.44	0.79	0.46	0.90	0.69
Rel sp act. of carbons of hexose-P and pentose-P						
C-1	17	94	20	100	3.6	14
C-2	100	100	100	86	100	100
C-3	14	8.6	15	7.6	8.0	4.5
C-4	2.4	61	2.4	51	1.5	55
C-5	17	10	16	10	18	2.0
C-6	2.9		3.2		0.7	

[a] The rates are expressed in micromoles per unit time relative to the rate of glucose utilization which is set as 100 μmoles/unit time. [b] Phosphoribosyl pyrophosphate. [c] Specific activity of carbon of highest activity. The specific activity of C-1 of glucose-1-^{14}C or C-2 of glucose-2-^{14}C is taken as 1.0. The relative specific activities are calculated on the basis of no endogenous dilution of the hexose-6-P or pentose-5-P.

found which agreed sufficiently close to the experimental ratios. Alternately, a computer program was set up for finding such values.[8]

(*A*) *Bacteria.* Sable and co-workers (Szynkiewiz *et al.*, 1961; Wright *et al.*, 1961; Sable and Cassisi, 1962) have studied synthesis of ribose and desoxyribose by *E. coli*, grown on labeled precursors, in a variety of conditions. They observed that the labeling pattern is variable, depending on the carbon source in the medium, the stage of growth, and the previous history of the culture. They concluded that ribose and desoxyribose have a common origin in this organism.

In Table II the labeling from glucose-2-^{14}C in glucose from bacterial polysaccharide and in ribose from RNA is presented. A first approximation for *PC* and *A* is

[8] A program to evaluate the parameters by means of least-squares estimates was set up for us by Dr. W. E. Smith of the Health Science Computing Facility at U.C.L.A. Details of the computer program are available from the authors.

TABLE II: Labeling by Glucose-2-^{14}C of Polysaccharide Glucose and RNA Ribose by *E. coli*.

	Expt[a] 1 Slow Growth		Expt[a] 2 Log Growth		Expt[a] 3 Stationary Phase		Expt[a] 4 Moderate Growth		Expt[a] 5 Log Growth		Expt[a] 6 Resting Stage	
	Glu	Rib	Glu	Rib	Glu	Rib	Glu	Rib	Glu	Rib	Glu	Rib
^{14}C in Carbon												
1	4.1	100	15	100	24	100	5.2	100	27	100	4.5	100
2	100	107	100	53	100	150	100	22	100	107	100	30
3	3.8		9.7		17		3.7		18		4.2	
5 or 4[b]	4.4	74	7.8	48	24	97	3.8	34	18	52	3.6	52
First Approximation[c]												
PC (%)		2		9		16		3		19		2
A		0.06		0.09		0.9		0.05		0.5		0.08
Computer Calculated[d]												
PC (%)		2		8		13		3		16		2
A		0.06		0.06		0.71		0.04		0.33		0.04
K		0.05		0.12		0.46		0.02		0.35		0.01
	Calculated Values for *PC*, *A*, and *K*[d]											
^{14}C in Carbon												
1	4.6	100	15	100	21	100	5.8	100	26	100	3.9	100
2	100	117	100	65	100	155	100	43	100	99	100	30
3	3.9		9.4		20		3.4		20		2.3	
5 or 4[b]	4.5	70	7.8	42	23	89	3.8	28	17	57	3.0	19

[a] Experiments 1–5 correspond to the tables of Szynkiewiz *et al.* (1961); expt 6, Sable and Cassisi (1962). [b] 5 for glucose, 4 for ribose. [c] $PC = (H_1/H_2)/2(1 - H_1/H_2)$; *A* from eq 17. [d] See Appendix B and footnote 8. Experiment 6 calculated by trial and error.

TABLE III: Labeling of DNA-Bound Glucose and DNA Deoxyribose by Phage T_2H of *E. coli*.

	Glucose-1-^{14}C[a]		Glucose-2-^{14}C[a]		Glucose-2-^{14}C[b]	
	Phage Glucose	Deoxyribose	Phage Glucose	Deoxyribose	Phage Glucose	Deoxyribose
^{14}C in carbon						
1	100	100	11	100	15	100
2	1.2	3.0	100	55	100	37
3	2.0	—	9.2	—	10	—
4	—	0	0	43	—	53
5	—	84	9.8	—	15	—
6	14	—	—	—	—	—
First approximation[c]						
PC(%)			7			9
A			0.3			0.5
Calculated[d]						
PC			7			8
A			0.15			0.46
K			0.07			0.05
Distribution in carbon						
1	100	100	13	100	14	100
2	0.4	6.8	100	58	100	56
3	2.8	—	8.4	—	10	—
4	—	—	—	38	—	42
5	—	80	9.7	—	17	—
6	9.7	—	—	—	—	—

[a] Wright *et al.* (1961). [b] Sable and Cassisi (1962). [c] As in Table II. [d] By trial and error for the experiment of footnote *a*. By computer (see footnote 8) for experiment of footnote *b*.

presented followed by the values of *PC*, *K*, and *A* as obtained by computer which provide the best agreement with the experimental results. Finally the theoretical ^{14}C patterns in hexose and pentose for these values of *PC*, *A*, and *K* are presented.

The agreement between the observed and calculated ^{14}C ratios is in most cases within the experimental error. In two experiments (4 and 6) the agreement between calculated and observed ratios in pentose is only fair. In these two experiments with resting cells the values of *PC* and *K* are very low, of the order of 0.01–0.03. Under these conditions changes of small magnitude cause marked alterations of ratios. Closer agreement between calculated and observed ratios may be obtained by considering a more complex model, with additional parameters.

In Table III results are summarized as reported by Wright *et al.* (1961) and Sable and Cassisi (1962) with *E. coli* infected with bacteriophage strain T_2H. This phage contains DNA-bound glucose. The use of glucose and desoxyribose from phage is of special interest as it is certain that they were formed synchronously. In bacteria, on the other hand, polysaccharide and RNA may have been formed at different phases of growth.

In Table IV the values of *PC*, *K*, and *A* for *E. coli* were converted into relative rates, expressed as μmoles of substrate/100 μmoles of glucose utilized by the bacteria. If the actual rates of glucose utilization were available, the steady-state rates could be expressed in absolute units (such as micromoles per hour per gram or other units).

(*B*) *Regenerating Liver.* Horecker *et al.* (1958) injected glucose-2-^{14}C into a partially hepatectomized rat (90% of the liver removed) every 6 hr for 56 hr. The liver was removed 68 hr after hepatectomy, and glycogen, RNA, and DNA were isolated. Glucose, ribose, and desoxyribose from these compounds were degraded. The distribution of ^{14}C in carbons 1–3 and 5 of hexose and 1, 2, and 4 of pentose are summarized in Table IV.

By a procedure similar to that employed in the previous section, values of *PC*, *K*, and *A* were found which agreed best with the data. The parameters and the corresponding theoretical ratios are presented in Table V. Theory and observed values are fairly close. In regenerating liver the contribution of the pentose cycle appears to be about 20%, and the rates of transketolase and transaldolase are roughly equal to the rate of glucose utilization.

It is doubtful whether all the assumptions for eq 1–15 are satisfied in this experiment. Thus steady-state conditions may not have prevailed and glycogen, RNA, and DNA may have been formed at different stages of regeneration. However, the agreement between observed

TABLE IV: Relative Rates of Glucose-6-P Dehydrogenase, Transaldolase, and Transketolase in *E. coli* and Bacteriophage under Several Conditions.

	Slow Growth[a]	Stationary Phase[b]	Log Growth[c]	Log Growth[d]	Phage T_2H[e]
PC (%)	2	13	8	16	8
A	0.05	0.7	0.06	0.3	0.3
K	0.03	0.5	0.1	0.3	0.07
			Relative rates (moles/unit time)		
Glucose utilization	100	100	100	100	100
Glucose-6-P decarboxylation	6	39	24	48	24
Phosphofructokinase	98	87	92	84	92
Transaldolase[f]	7	83	14	46	38
	5	70	6	30	30
Transketolase[g]	8	113	28	76	22
	6	100	20	60	14

[a] Average of expt 1 and 4 of Szynkiewiz *et al.* (1961) and expt 1 of Sable and Cassisi (1962). [b] Experiment 3 of Szynkiewiz *et al.* (1961). [c] Experiment 2 of Szynkiewiz *et al.* (1961). [d] Experiment 5 of Szynkiewiz *et al.* (1961). [e] Average of Table III. [f] The top figure, rate is in the forward direction of Figures 2 and 3. Sedoheptulose is the substrate. The bottom figure, rate in the reverse direction. [g] It is assumed that the two transketolase reactions are equal, and the forward rate (Figure 2) is expressed in terms of xylulose-5-P. Top forward rate, bottom reverse rate.

TABLE V: Distribution of ^{14}C from Glucose-2-^{14}C in Glycogen, and RNA and DNA Pentose from Regenerating Rat Liver.[a]

	Observed Ratios				
	H_1/H_2	H_3/H_2	H_5/H_2	P_2/P_1	P_4/P_1
Glycogen glucose	0.35	0.21	0.28		
RNA ribose				1.5	0.53
DNA deoxyribose				1.0	0.44
Parameters	Calculated				
$PC = 22\%$, $A = 0.98$, $K = 0.65$	0.31	0.29	0.26	1.3	0.76
$PC = 23\%$, $A = 1.14$, $K = 0.50$	0.32	0.26	0.26	0.89	0.53

[a] Data of Horecker *et al.* (1958).

and calculated ^{14}C distribution lends support to the major features of the model postulated by us.[9]

Muscle. Green and Landau (1965) incubated heart, abdominal, and diaphragm muscle of rats with glucose-2-^{14}C and isolated and degraded glucose from glycogen and ribose from nucleotides. The ^{14}C recovery in ribose was very low. The randomization of ^{14}C in C-1 and C-3 of glucose was rather limited, but there was appreciable ^{14}C in C-5 of glucose, about 20% that in C-2. There was roughly equal labeling of C-1 and C-2 of ribose, the P_2/P_1 ratio ranging from 0.5 to 1.2. Typical results and estimates of *PC*, *K*, and *A* are presented in Table VI. It appears that in muscle the pentose cycle contribution is rudimentary (less than 0.5%) and transketolase activity is also low, but the rate of transaldolase high. Comparison of these estimates with rates measured by conventional enzyme assay in muscle extracts would be of interest.

The very low yield of ^{14}C in ribose calls for caution in interpretation. It is possible that the nucleotides are formed by only some specialized cells in the muscle and the labeling pattern in glycogen and ribose were not derived from a single glucose-6-P pool.

HeLa Cells. Hiatt (1957a) grew HeLa cells for 6 days with glucose-1-^{14}C, -2-^{14}C, and ribose-1-^{14}C. RNA ribose was isolated and degraded. The ^{14}C labeling of the major carbons is shown in Table VII. No glycogen

[9] In these experiments there was high randomization in C-3 of pentoses and C-4 of hexose (see Table II, Horecker *et al.*, 1958). No satisfactory explanation for the randomization in these positions is apparent.

TABLE VI: Distribution of ^{14}C in Glucose of Glycogen and Ribose of Nucleotide from Muscle of Baby Rats Incubated with Glucose-2-^{14}C.[a]

	H_1/H_2	H_3/H_2	H_5/H_2	P_2/P_1
Heart[a]	0.02	0.04	0.24	0.60
Diaphragm[b]	0.01	0.02	0.20	1.2
		Calculated[c]		
$PC = 0.5\%$, $K=0.005$, $A = 0.70$	0.01	0.01	0.21	0.55
$PC = 0.5\%$, $K = 0.01$, $A = 0.70$	0.01	0.01	0.21	1.5

[a] Average of expt 2 and 3 of Table I (Green and Landau, 1965). [b] Experiment 1. [c] By trial and error.

TABLE VII: HeLa Cells Incubated for 6 Days with Glucose-1-^{14}C, Glucose-2-^{14}C, and Ribose-1-^{14}C.[a]

	Obsd Act. of RNA Ribose from		
Carbon	Glucose-1-^{14}C	Glucose-2-^{14}C	Ribose-1-^{14}C
1	100	100	100
2	8.7	175	5.3
4	—	104	—
5	78	—	14
Calculated[b] for 5% Pentose Cycle Contribution, $K = 0.20$, $A = 0.70$			
P_2/P_1	0.095	1.8	0.05
P_4/P_1 or P_5/P_1	0.75	0.95	0.10

[a] Data of Hiatt (1957a). The ribose from RNA was isolated and degraded and the specific activity of carbons was determined. [b] Trial and error.

was formed in these cells. The data represent six ratios, in theory more than necessary to estimate all parameters. The use of ribose as a substrate required minor modifications of eq 1–15. Since in the absence of glycogen it is not possible to obtain a first approximation for *PC* and *A*, the evaluation of the parameters by trial and error is very tedious. A set of values of 5% *PC*, $K = 0.2$, and $A = 0.70$ was found to yield ratios resembling the experimental ones. These are presented at the bottom of Table VII. While the agreement seems fairly close, the estimation of the parameters is uncertain, since fair agreement may be obtained by other combinations of the three parameters. An estimation of the pentose cycle by using yields from glucose-1-^{14}C and -6-^{14}C in CO_2 and lactate used in conjunction with the data of Table VII would make the evaluation of the parameters much more reliable.

Proportion of Glucose Converted to RNA Pentose. The fraction of glucose carbon recovered in RNA ribose was not reported in the experiments of Sable and co-workers (Szynkiewiz *et al.*, 1961; Wright *et al.*, 1961; Sable and Cassisi, 1962). However, such incorporation is not likely to exceed 2% of the utilized glucose.[10] In rapidly growing cultures, the contribution of the pentose cycle was about 15%. Thus per 1000 μmoles of glucose utilized, 470 μmoles ([(3 × 0.15) + 0.02]1000) of ribose-5-P would be formed from glucose-6-P, of which 450 would recycle to fructose-6-P and about 20 would be incorporated into phosphoribosyl pyrophosphate and RNA ribose. This rate of synthesis of PRPP ($R = 0.02$) would not affect significantly the ^{14}C distribution in the phosphate esters and the ratios of Tables II and III (see Figure 8).

In mammalian tissues, the fraction of glucose carbon incorporated in the pentose of nucleic acids and nucleotides is much less than in microorganisms. In the experiments with regenerating liver, reported above, only 0.02% of the injected glucose-^{14}C was recovered in isolated liver nucleates (Horecker *et al.*, 1958). With an estimated contribution of the pentose cycle of 20% (Table IV), well over 99% of the ribose-5-P would be recycled to fructose-6-P. Even if 1% of the glucose was used for RNA ribose synthesis, only about 1 out of 60 molecules of ribose-5-P would be converted to PRPP.

In rats and mice injected with glucose-^{14}C, recovery of ^{14}C in nucleic acids is about 0.1–0.2% (Hiatt, 1957b; Vrba, 1964). The synthesis of pentose-5-P is greatly augmented in animals treated with imidazoleacetic acid. This acid is conjugated with ribose and excreted in urine as a riboside. Hiatt (1957b) recovered up to 0.5% of the injected glucose in the riboside. Assuming that the contribution of the pentose cycle to glucose metabolism *in vivo* is as low as 1%, we find that one-sixth of the pentose-5-P is converted to PRPP and a much lower conversion is likely in untreated animals.

In muscle *in vitro*, Green and Landau (1965) found the incorporation of glucose-^{14}C into ribose of nucleotides and nucleic acids very low. Even with a contribution of the pentose cycle of 0.1%, very little of the ribose-5-P would be converted to PRPP.

In embryonic tissue the utilization of glucose for ribose and desoxyribose synthesis is likely to be relatively high. Available data indicate that even in this tissue most of the ribose-5-P is not converted to PRPP. Crockett and Leslie (1963) established a complete carbon balance in a line of foetal cells grown in culture. They found less than 3% of utilized ^{14}C in total RNA and DNA and the contribution of the pentose cycle to be about 7%.[11] This indicates recycling of at least 90% of the pentose-5-P. Thus in mammalian tissue and *E. coli* the fraction of ribose-5-P incorporated into phospho-

[10] *E. coli* contains about 15% RNA and DNA on a dry weight basis (Luria, 1960) or about 6% as pentose. Assuming 3 g of glucose carbon is metabolized in the synthesis of 1 g of cell carbon the conversion of glucose to pentose is 2%. (See also footnote 1 of Wood *et al.* (1963) where it is estimated that about 1% of utilized glucose carbon appears in nucleic acids of a number of anaerobically grown organisms.)

ribosyl pyrophosphate is too small to evaluate from ^{14}C randomization. The fraction of utilized glucose carbon used for nucleic acid pentose synthesis is best estimated by measuring the ^{14}C yield (preferably from glucose-U-^{14}C) into these compounds.

Estimation of Other Rates. Availability of five ratios permits, in theory, evaluation of additional rates. In several experiments, the possibility of obtaining closer agreement between observed and calculated ^{14}C distribution was explored by varying independently the two transketolase rates (K_1 and K_2, Figure 2). No significant improvement in fit was obtained. It seems that the two transketolase rates are similar and vary at most by 20% from each other.

So far the pentose phosphates were considered to be completely equilibrated. Lack of equilibration would not greatly alter the ^{14}C ratios in hexose, but would change considerably the ratios in pentose. In most experiments, the agreement between calculated and observed ratios in ribose was sufficiently close to indicate extensive equilibration of pentose phosphates. In a few experiments the possibility of obtaining better fits by varying the rates of epimerization and isomerization of pentose phosphates was explored. A computer program based on 21 equations (see Appendix) was set up for this purpose. There was little improvement in the agreement between experimental data over those calculated from a simpler model.

It appears that solution of systems with more than three parameters (*PC*, *K*, and *A*) requires extensive experimental data, such as the simultaneous use of glucose-1-^{14}C, -2-^{14}C, and -6-^{14}C as substrate, or better yet isolation of several pentoses or sedoheptulose. Such systems cannot be handled by trial and error and require development of special computer programs.

Discussion

Determination of Steady-State Rates. Conventional methods of enzyme assay measure activities under optimal conditions, in the presence of excess substrate, when kinetics is usually zero order. Such activities may bear little relationship to actual rates in intact tissue. Methods for determination of steady-state rates of readily reversible reactions have not been generally available. Procedures, applicable in the presence of the pentose cycle, for evaluation of such steady-state rates have been developed by us for the isomerization of hexose and triose phosphates (Katz and Wood, 1960; Landau *et al.*, 1964). Now such procedures have been developed for transaldolase and transketolase, and in principle for isomerization and epimerization of pentose phosphates. Comparison of steady-state rates of such reversible reactions with enzymatic activities measured in cell-free extracts would be of interest.

Recycling of Pentose-5-P and Synthesis of Phosphoribosyl Pyrophosphate. Two points of view on the synthesis of ribose-5-P have been advanced. We (Wood *et al.*, 1963) have based our interpretation and calculations on the assumption that the oxidative formation of pentose-5-P exceeds greatly its utilization for synthesis, and that the bulk of pentose-5-P is recycled to fructose-6-P. An opposite view is expressed by Horecker (1965): "...the bulk of the label from glucose-2-^{14}C is found in atom 2 of ribose..., this pentose must, therefore, have been formed largely by the nonoxidative pathway. In most mammalian cells about 75% of the ribose phosphate arises by this pathway. It may therefore be concluded that the C-1 oxidation does not operate as a cycle but rather as two parallel mechanisms for the formation of pentose-5 phosphate from hexose-6 phosphate." The conclusion that 75% of the ribose phosphate arises by the nonoxidative pathway is based on incorporation of ^{14}C without taking in account the reversibility of the system. ^{14}C incorporation does not always represent the *net* flow of carbon. We have shown that the exchange of ^{14}C into the pentose-P *via* the reversible transketolase and transaldolase reactions is high but the *net* flow of carbon, nevertheless, is from pentose-P to fructose-6-P in the nonoxidative pathway. Thus the pentose pathway is a cycle and not two pathways for making pentose-P. The distribution of ^{14}C in pentose is fully consistent with our postulates, but not with the model in Figure 4 in which the pentose pathway does not serve as a cycle. The data indicate in a variety of mammalian tissues and *E. coli* (and probably other bacteria of similar type) that the outflow from the pentose-5-P pool for synthesis of PRPP is minor. In these tissues and bacteria the major quantitative role of the pentose cycle is the generation of reduced triphosphopyridine nucleotide, required for reductive biosynthesis. Synthesis of nucleic acids and nucleotides is of course a process of utmost importance, but it requires a small fraction of the ribulose-5-P formed in the pentose cycle.

It is possible that in some classes of bacteria and in plants synthesis of derivatives of sedoheptulose or erythrose plays an important role (shikimic acid, persitol, and others). No data on the quantitativesignificance of these reactions are available. In some groups of bacteria, such as clostridia and pseudomonads, glucose-6-P dehydrogenase is absent (Horecker, 1962) and pentose phosphate synthesis probably proceeds solely by the nonoxidative pathway. It is likely that some microorganisms will be found which contain little glucose-6-P dehydrogenase and which synthesize pentose-5-P by a combination of the oxidative and nonoxidative pathways. However, this type of synthesis has not yet been described.

Rates of Transaldolase and Transketolase. While the data are limited they suggest that in *E. coli* there is some

[11] These workers grew a strain of human foetal liver cells in the medium of Waymouth for 48 hr with glucose-1-^{14}C, -6-^{14}C, and -U-^{14}C and determined the amount of glucose utilization. A complete carbon balance was established and reliable determination of the pentose cycle is possible from the data. From specific $^{14}CO_2$ yields and lactate ratios, values of 7.2 and 7.7%, respectively, are obtained. The incorporation in RNA and DNA was 2.8% of the utilized glucose-U-^{14}C. Assuming that the ^{14}C is only in pentose, per mole of glucose used about 250 mmoles of pentose-5-P was formed and 30 was converted to PRPP. This value is minimal since some of ^{14}C in nucleic acids is likely to be present in purines and pyrimidines.

TABLE VIII: Illustration of Errors in the Calculation of the Pentose Cycle Due to Neglecting the Effects of Reversible Transaldolase and Transketolase.[a]

Labeled Sugar	Determination	Value when $K = A = 0$	Value when $K = A = 0.5$	Apparent Pentose Cycle %
Glucose-1-^{14}C[b]	Specific $^{14}CO_2$ yield from glucose-1-^{14}C *via* the pentose cycle	25%	22%	8.5
Glucose-1-^{14}C Glucose-6-^{14}C	Ratio of yields in fatty acids	0.75	0.69	13
Glucose-1-^{14}C Glucose-6-^{14}C	Ratio of yields in lactate	0.75	0.77	9
Glucose-2-^{14}C	H_1/H_2 ratio in glycogen	0.167	0.182	13
Glucose-2-^{14}C	H_3/H_2 ratio in glycogen	0.091	0.184	22.5

[a] Errors are calculated for a case when the contribution of the pentose cycle is 10% and $A = 0.5$ and $K = 0.5$.
[b] Yield of $^{14}CO_2$ from glucose-6-^{14}C very small.

relationship between the rates of glucose-6-P dehydrogenase, transaldolase, and transketolase. When the level of the pentose cycle is high all three enzymes show high activities. The data in mammalian tissues are too few to permit generalization.

Transketolase and transaldolase affect markedly the ^{14}C distribution in the intermediates, and these reactions are significant in the interpretation of ^{14}C patterns, especially in pentoses. In the cases examined here these rates are higher than those required for the operation of the pentose cycle.

Estimation of the Pentose Cycle. Determination of pentose cycle contribution is of physiological interest, since it is essential in establishment of pyridine nucleotide balance and energy balance in cells, as has been shown in adipose tissue (Rognstad and Katz, 1966). Procedures used previously for the calculation of the pentose cycle were based on a model which assumed unidirectional operation of transketolase and transaldolase. The direction and magnitude of the errors that may be caused by this simplifying assumption are illustrated in Table VIII. The apparent values obtained for the pentose cycle contribution by several methods have been calculated for a pentose cycle of 10% and when A and K equal 0.5. Procedures based on yields from glucose-1-^{14}C and -6-^{14}C and the H_1/H_2 ratio in glycogen from glucose-2-^{14}C give values which deviate from the true ones by -15 to $+30$%. Calculations based on the H_3/H_2 ratio from glycogen lead to a very large overestimate, more than double the correct figure. If K and A equaled 0.1, the apparent pentose cycle calculated from the H_3/H_2 ratio would be 11%. Other methods would show no significant difference from the true value of 10%.

It seems that reasonable estimates of the pentose cycle are frequently possible even when the effect of reversibility of transketolase–transaldolase is neglected. As seen in Tables II–IV, the approximation of the pentose cycle from the H_1/H_2 ratios is usually adequate. However the H_3/H_2 ratio should be used with caution.

Validity of the Model. The present model is much less restricted than those used previously. It still contains many assumptions, including that of isotopic and metabolic steady state, and may not be adequate for all systems. The equations offer predictions for the ^{14}C distribution in a variety of compounds, and this lends itself to experimental test of their validity. The agreement between theory and experimental data seems reasonable in *E. coli* and a number of mammalian tissues reviewed here. The theory explains adequately the labeling pattern in hexose and pentose formed from glucose-^{14}C. It accounts for the ^{14}C distribution in the top and bottom carbons of these sugars. No plausible explanations for the labeling pattern of the pentoses have been available hitherto. The agreement between theoretical and observed ^{14}C patterns lends support to the validity of the model and the correctness of our analysis.

Appendix

(*A*) *Derivations of Eq 1–15.* A general and simple procedure for deriving these equations is based on the expressions of Figure 2. Expressions I–III represent reversible reactions catalyzed by transketolase or transaldolase. Expressions IV–VII represent irreversible reactions for outflow from the hexose-6-P, pentose-5-P, and triose-P pools. For each position (H_2, P_5, S_4, etc.) there is one or more outflows. The inflows and outflows are readily obtained by inspection of Figures 2 and 3. In the steady state, total inflow equals total outflow, and the equations are obtained by summing up inflow and outflow for each position.

In addition to expressions I–VII the inflow of ^{14}C from the substrate must be considered. For glucose-1-, -2-, or -3-^{14}C there is a sole inflow, respectively into positions 1, 2, or 3 of hexose-6-P. The inflow from the substrate glucose is represented by 1, which appears in equations for H_1, H_2, or H_3, respectively. Otherwise the equations for these three labeled glucoses are identical. The tech-

SCHEME III

	Inflow			Outflow		
Position	Expression	From	Rate	Expression	Rate	Eq
H_1	II	P_1	$PC + K_2$	II	K_2	
	III	S_1	$PC + A$	III	A	
		Substrate	1	IV	$3PC + R$	
				V	$1 - PC - R$	
	$1 + P_1(PC + K_2) + S_1(PC + A)$			$= H_1(1 + 2PC + K_2 + A)$		1
H_3	II	E_1	$PC + K_2$	II	K_2	
	III	S_3	$PC + A$	III	A	
				IV	$3PC + R$	
				V	$1 - PC - R$	
	$E_1(PC + K_2) + S_3(PC + A)$			$= H_3(1 + 2PC + K_2 + A)$		3
P_2	I	S_2	K_1	I	$PC + K_1$	
	I	S_4	K_1	I	$PC + K_1$	
	II	H_2	K_2	II	$PC + K_2$	
	IV	H_3	$3PC + R$	VII	R	
	$K_1(S_2 + S_4) + H_2K_2 + H_3(3PC + R)$			$= P_2(3PC + 2K_1 + K_2 + R)$		5
P_5	I	S_7	K_1	I	$PC + K_1$	
	I	Tr_3	K_1	I	$PC + K_1$	
	II	Tr_3	K_2	II	$PC + K_2$	
	IV	H_6	$3PC + R$	VII	R	
	$S_7K_1 + Tr_3(K_1 + K_2) + H_6(3PC+R)$			$= P_5(3PC + 2K_1 + K_2 + R)$		12
Tr_3	I	P_5	$PC + K_1$	I	K_1	
	II	P_5	$PC + K_2$	II	K_2	
	III	H_6	A	III	$PC + A$	
	V	H_1	$1 - PC - R$	VI	$2 - PC - 2R$	
	V	H_6	$1 - PC - R$			
$P_5(2PC + K_1 + K_2) + H_6(1 - PC + A - R) + H_1(1 - PC - R)$				$= Tr_3(2 + K_1 + K_2 + A - 2R)$		15

nique for deriving eq 1–15 is illustrated in detail for glucose-1-^{14}C as substrate in the examples in Scheme III.

Nonequilibration of Pentose Phosphates. When ribulose 5-P (Ru), xylulose-5-P (Xu), and ribose-5-P (Ri) are not equilibrated, two new parameters are introduced, EP for epimerase, and IS for isomerase. These are defined in the same way as K and A. Expressions I–VI are analogous to I–VI of Figure 2. Two new expressions, VIII and IX, are introduced.

$$Xu + Ri \underset{K_1}{\overset{PC + K_1}{\rightleftharpoons}} S + T \qquad I$$

$$Xu + E \underset{K_2}{\overset{PC + K_2}{\rightleftharpoons}} H + T \qquad II$$

$$S + T \underset{A}{\overset{PC + A}{\rightleftharpoons}} H + E \qquad III$$

$$H \xrightarrow{3PC} CO_2 + Ru \qquad IV$$

$$H \xrightarrow{1 - PC} 2Tr \qquad V$$

$$Tr \xrightarrow{2 - PC} \qquad VI$$

$$P \xrightarrow{R} PRPP \qquad VII$$

$$Ru \underset{\leftarrow}{\overset{\rightarrow}{EP}} Xu \qquad VIII$$

$$Ru \underset{\leftarrow}{\overset{\rightarrow}{IS}} Ri \qquad IX$$

From these expressions a set of 21 equations is derived by the procedure outlined for eq 1–15. The equations and computer decks for their solution are available from the authors upon request.

Synthesis of Pentose by the Oxidative and Nonoxidative Pathways. Expressions for this condition are based on the model of Figure 4B. R is the rate of pentose-5-P formation through decarboxylation of phosphogluconate, and T *via* transaldolase and transketolase. The rate of PRPP synthesis is $R + T$. The reactions are the same as those of Figure 2, but the rates differ. The revised expressions are numbered I–VII, paralleling those of Figure 2. For simplicity in these expressions the two rates of transketolase are taken to be equal ($K_1 = K_2$).

SCHEME IV

Ester	Position	Inflow		Outflow	Eq
Hexose-6-P	H_1	$1 + KP_1 + AS_1$	=	$H_1(1 + K + A)$	1′
	H_2	$KP_2 + AS_2$	=	$H_2(1 + K + A)$	2′
	H_3	$KE_1 + AS_3$	=	$H_3(1 + K + A)$	3′
	H_6	$KE_4 + ATr_3$	=	$H_6(1 + K + A)$	11′
Pentose-5-P	P_1	$RH_2 + (H_1 + S_1 + S_3)(K + (T/3))$	=	$P_1(3K + R + T)$	4′
	P_2	$RH_3 + (H_2 + S_2 + S_4)(K + (T/3))$	=	$P_2(3K + R + T)$	5′
	P_5	$RH_6 + (2Tr_3 + S_7)(K + (T/3))$	=	$P_5(3K + R + T)$	12′
Sedoheptulose-7-P	S_1	$KP_1 + H_1(A + (T/3))$	=	$S_1(K + A + T/3)$	6′
	S_2	$KP_2 + H_2(A + (T/3))$	=	$S_2(K + A + (T/3))$	7′
	S_3	$KP_1 + H_3(A + (T/3))$	=	$S_3(K + A + (T/3))$	8′
	S_4	$KP_2 + E_1(A + (T/3))$	=	$S_4(K + A + (T/3))$	9′
	S_7	$KP_5 + E_4(A + (T/3))$	=	$S_7(K + A + (T/3))$	13′
Erythrose-4-P	E_1	$AS_4 + H_3(K + (T/3))$	=	$E_1(K + A + (T/3))$	10′
	E_4	$AS_7 + H_6(K + (T/3))$	=	$E_4(K + A + (T/3))$	14′
Triose-P	Tr_3	$2KP_5 + H_6(1 + A - R - (T/3)) + H_1(1 - R - (^2/_3T))$	=	$Tr_3(2 + 2K + A - 2R - T)$	15′

$$P + P \underset{K + T/3}{\overset{K}{\rightleftharpoons}} S + Tr \qquad \text{I}$$

$$P + E \underset{K + T/3}{\overset{K}{\rightleftharpoons}} H + Tr \qquad \text{II}$$

$$S + Tr \underset{A + (T/3)}{\overset{A}{\rightleftharpoons}} H + E \qquad \text{III}$$

$$H \xrightarrow{R} CO_2 + P \qquad \text{IV}$$

$$H \xrightarrow{1 - R - (^2/_3T)} Tr \qquad \text{V}$$

$$Tr \xrightarrow{2 - 2R - (^5/_3T)} \qquad \text{VI}$$

$$P \xrightarrow{R + T} \qquad \text{VII}$$

Equations based on these expressions with glucose-1-^{14}C as substrate are derived using the procedure described before. The equations are numbered 1′–15′ (Scheme IV) to correspond with eq 1–15.

(*B*) *Solution of the Equations by Computer.*[12] The program for solutions of the equations was set up in FORTRAN 2 language, used in conjunction with a 7090 IBM computer. The program consists of a main routine and three subroutines; the main routine reads in the known parameters (*PC*, K_1, K_3, *A*, *R*, etc.) and the constant vector. (The constant vector is determined by whether the substrate is glucose-1-^{14}C, -2-^{14}C, or -3-^{14}C, or ribose-1-^{14}C and -2-^{14}C.)

The main routine also varies the *PC* value from P_0 up to P_{max} at the desired interval. The other parameters are also varied at the desired range. Then for each set of parameters the main routine calls the following subroutines in order to solve the problem: (1) subroutine A sets up the *n* linear equations which are to be solved; (2) subroutine B solves the set of *n* linear equations and prints out the solution for the unknowns; (3) subroutine C calculates the proper ratios and prints out their values.

The main routine also prints out the known parameters and the constant vector. It is possible to solve the set of equations with different constant vectors and the same set of parameters. The FORTRAN decks for eq 1–15, eq 1′–15′, and the set of 21 equations when pentose phosphates are not equilibrated are available from the authors upon request.

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[12] The authors wish to acknowledge the assistance of Mr. H. Rostami, who set up this program and instructed the authors in its use.

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Biosynthesis of Nitro Compounds. II. Studies on Potential Precursors for the Nitro Group of β-Nitropropionic Acid*

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ABSTRACT: The incorporation of ^{15}N- and ^{18}O-labeled substrates into β-nitropropionic acid by growing cultures of *Penicillium atrovenetum* was investigated. Analyses of the β-nitropropionic acid samples by mass spectrometry of their methyl esters indicated that ammonium ion was used for the synthesis of the nitro group in preference to nitrate. The label from [^{18}O]potassium nitrate was not incorporated into the nitro group. The amino group of aspartic acid was utilized in preference (*ca.* 2: 1) to ammonium ion for the synthesis of the nitro group. [3-^{14}C]- and [4-^{14}C]-aspartic acids were incorporated equally well into β-nitropropionic acid. Dilution of the label was small in spite of a low efficiency of incorporation. L-[4-^{14}C]-Aspartic acid, but not the corresponding D isomer, was incorporated into the nitro compound. It was concluded that both the amino group and the carbon skeleton of aspartic acid are on a direct pathway to β-nitropropionic acid.

Label from tartaric acid, which promotes β-nitropropionic acid synthesis, was not incorporated into the nitro compound.

Previous evidence indicated that a reduced nitrogen compound was required by *Penicillium atrovenetum* for the synthesis of β-nitropropionic acid (Raistrick and Stössl, 1958; Hylin and Matsumoto, 1960; Shaw and Wang, 1964). Results reported by these workers suggested that ammonium ion was the obligatory precursor of the nitro group. Birch *et al.* (1960), on the other hand, proposed that aspartic acid was a direct precursor of β-nitropropionic acid and, furthermore, that the aspartic acid amino group was oxidized *in situ* to the nitro group. This view was supported by results given in a brief report by Gatenbeck and Forsgren (1964) who studied the incorporation of [^{15}N,U-^{14}C]-aspartic acid into β-nitropropionic acid.

In an attempt to resolve the question of the source of the nitro group of β-nitropropionic acid, a series of experiments was undertaken on the utilization of various labeled nitrogen compounds by *P. atrovenetum*. The results of these experiments are the substance of this communication.

Materials and Methods

Labeled compounds were obtained from the following sources: [^{15}N]potassium nitrate, Volk Radiochemical Co.; [^{15}N]ammonium chloride, Bio-Rad Laboratories and Nichem, Inc.; L-[^{15}N]aspartic acid, Merck Sharp & Dohme of Canada, Ltd., and Volk Radiochemical Co.; [^{18}O]potassium nitrate, Yeda Research and Development Co., Ltd.; DL-[3-^{14}C]-aspartic acid, Nuclear Research Chemicals, Inc.;

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