Biochem. J. (1978) **176**, 257–282
Printed in Great Britain

New Reaction Sequences for the Non-Oxidative Pentose Phosphate Pathway

By JOHN F. WILLIAMS, PETER F. BLACKMORE* and MICHAEL G. CLARK†
Department of Biochemistry, School of General Studies, Australian National University, Canberra, A.C.T. 2600, Australia

(*Received* 28 *March* 1978)

1. Reactions leading to the formation of ^{14}C-labelled volatile compounds and compounds volatile under acid conditions were investigated in a system actively synthesizing hexose 6-phosphates from [U-^{14}C]ribose 5-phosphate by reactions catalysed by enzymes prepared from acetone-dried powder of rat liver; no reactions involving ^{14}C-labelled volatile compounds were detected. Similarly the fixation of ^{14}C-labelled volatile compounds into hexose 6-phosphate could not be detected. 2. A complete carbon balance was made for the reactants, intermediates and products of the reactions involved in the conversion of ribose 5-phosphate into hexose 6-phosphate by enzymes of rat liver. Five additional intermediates of pentose 5-phosphate metabolism in liver were detected, namely D-*manno*-heptulose 7-phosphate, D-*altro*-heptulose 1,7-bisphosphate, D-*glycero*-D-*ido*-octulose 1,8-bisphosphate, D-*glycero*-D-*altro*-octulose 1,8-bisphosphate and D-arabinose 5-phosphate. 3. D-Arabinose 5-phosphate was found to be utilized by a rat liver enzyme preparation to produce both hexose 6-phosphate and triose phosphate. 4. D-Arabinose 5-phosphate was reversibly converted into other pentose 5-phosphates. Paper chromatographic and enzymic evidence indicated that the conversion involved an enzyme tentatively named arabinose phosphate 2-epimerase, which catalyses the following reaction: D-arabinose-5-*P* $\rightleftharpoons$ D-ribose-5-*P*. 5. A variety of rat tissues also utilized D-arabinose 5-phosphate to produce both hexose 6-phosphate and triose phosphate and at a rate comparable with that obtained with D-ribose 5-phosphate. 6. A new reaction sequence for the non-oxidative pentose phosphate pathway in liver is proposed.

The defined reaction mechanism of the non-oxidative pentose phosphate pathway is based on the results of two experiments, one using a rat liver enzyme preparation and the other using a pea root and pea leaf enzyme preparation (Horecker *et al.*, 1954; Gibbs & Horecker, 1954). The experiment of Horecker *et al.* (1954), which involved the degradation of [^{14}C]glucose 6-phosphate‡ formed from [1-^{14}C]ribose 5-phosphate after 17h incubation with a rat liver enzyme preparation, has been repeated and extended (Williams *et al.*, 1978). The distribution of ^{14}C in glucose 6-phosphate formed from [1-^{14}C]-ribose 5-phosphate by the rat liver enzyme preparation during the early time intervals of the incubation (Williams *et al.*, 1978) could not be reconciled with the operation of any of the recognized metabolic pathways involving non-oxidative pentose 5-phosphate–hexose 6-phosphate interconversions. Two markedly different rates of ribose 5-phosphate utilization were found to occur. The more rapid rate of ribose 5-phosphate utilization coincided with heavy labelling in C-2 and C-6 of glucose 6-phosphate, while the slower phase of ribose 5-phosphate utilization coincided with heavy labelling in C-1 and C-3 of glucose 6-phosphate. These results, particularly those involving different ^{14}C-labelling patterns, suggest the possibility of at least two different reaction pathways for the conversion of ribose 5-phosphate into hexose 6-phosphate by the rat liver enzyme preparation. Only the reactions which occur after 3h, in the incubation, are directed towards a ^{14}C distribution pattern originally found by Horecker *et al.* (1954).

Carbon-balance studies conducted during the 17h incubation of ribose 5-phosphate with the rat liver enzyme preparation (Williams *et al.*, 1978) indicated that a significant amount of the substrate ribose 5-phosphate carbon could not be accounted for in the intermediates currently assigned to the reaction scheme of the pentose phosphate pathway. The theoretical stoicheiometry of the non-oxidative pentose phosphate pathway states that 3mol of pentose 5-phosphate will form 2mol of hexose monophosphate and 1mol of triose phosphate (Wood & Katz, 1958).

* Present address: Department of Physiology, School of Medicine, Vanderbilt University, Nashville, TN 37232, U.S.A.

† Present address: Department of Biochemistry, Flinders Medical School, Flinders University of South Australia, Bedford Park, S.A. 5042, Australia.

‡ All sugars mentioned in this paper have the D-configuration unless otherwise stated.

In this present paper an alternative reaction scheme for the non-oxidative pentose phosphate pathway in liver is proposed which involves five additional intermediates and two new enzymes for mammalian liver metabolism. The concentrations of the five additional intermediates, arabinose 5-phosphate, D-*glycero*-D-*ido*-octulose 1,8-bisphosphate, D-*glycero*-D-*altro*-octulose 1,8-bisphosphate, *altro*-heptulose 1,7-bisphosphate and *manno*-heptulose 7-phosphate when considered together with the concentrations of recognized metabolites of the pentose phosphate pathway (Williams *et al.*, 1978) have accounted for 97% of the total substrate carbon. The stoicheiometry of pentose 5-phosphate utilization and of hexose 6-phosphate and triose phosphate formation at 17h for the new pathway is similar to that of the currently accepted reaction scheme for the pathway (Horecker *et al.*, 1954).

Experimental

Enzymes and chemicals

Enzymes and chemicals have been described in the preceding paper (Williams *et al.*, 1978). [^{14}C]-Formaldehyde, $NaH^{14}CO_3$, [^{14}C]formic acid sodium salt, [^{14}C]methanol, [U-^{14}C]glycerol, [U-^{14}C]glucose, [U-^{14}C]ribose, [U-^{14}C]glyoxylic acid (sodium salt), [1-^{14}C]glycollic acid (sodium salt), [U-^{14}C]acetic acid (sodium salt), [U-^{14}C]pyruvic acid (sodium salt) and [U-^{14}C]glucosamine hydrochloride were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. *altro*-Heptulose 7-phosphate, ribulose 1,5-bisphosphate and ribulose 1,5-bisphosphate carboxylase type I were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.)

Synthetic substrates

Arabinose 5-phosphate was prepared from glucosamine 6-phosphate as described by Volk (1966). Erythrose 4-phosphate was prepared from glucose 6-phosphate by the method of Simpson *et al.* (1966). The commercial *altro*-heptulose 7-phosphate, which was contaminated with hexose and pentose phosphates, was further purified, as were the preparations of arabinose 5-phosphate and erythrose 4-phosphate by ion-exchange chromatography by the method of Williams *et al.* (1971), followed by paper chromatography using solvent system C (see below).

Preparation of [^{14}C]substrates

[U-^{14}C]Ribose 5-phosphate was prepared from [U-^{14}C]glucose by the same method described for the preparation of [1-^{14}C]ribose 5-phosphate from [2-^{14}C]glucose in the preceding paper (Williams *et al.*, 1978). [U-^{14}C]Erythrose 4-phosphate was prepared from [U-^{14}C]glucose 6-phosphate by the method of Simpson *et al.* (1966). [U-^{14}C]Arabinose 5-phosphate was prepared from [U-^{14}C]glucosamine 6-phosphate as described by Volk (1966). Both the [U-^{14}C]erythrose 4-phosphate and [U-^{14}C]arabinose 5-phosphate were purified as described above for the unlabelled sugar phosphates.

Paper-chromatographic methods

Analytical and preparative paper chromatography was carried out on Whatman 3MM chromatography paper. The following solvent systems were used: (A) ethyl acetate/pyridine/water (12:5:4, by vol.), ascending for the separation of free sugars; (B) butan-1-ol/acetic acid/water (10:21:50, by vol.), descending for the separation of free sugars; (C) the GW_3 solvent described by Wood (1968), ascending and run twice in the same direction, for the separation of sugar phosphates; (D) 80% ethanol containing 0.64% boric acid for the separation of ribose 5-phosphate and arabinose 5-phosphate, as described by Cohen & McNair Scott (1950). The positions of sugars and sugar phosphates were located on chromatograms by either scanning for radioactivity in a Nuclear–Chicago Actigraph III Radioscanner and/or by staining the chromatogram with the aniline/diphenylamine stain described by Smith (1958).

Enzyme preparations

The rat liver enzyme preparation was prepared by the method described in the previous paper (Williams *et al.*, 1978). The other enzyme preparations for the various tissues of the rat were prepared by extracting the appropriate acetone-dried powder of the tissue in 12 vol. of 0.25M-glycylglycine/KOH buffer for 15s, in a Potter–Elvehjem homogenizer, and centrifuging at 10000*g* for 10min. The supernatant was then used for the enzyme studies.

Analytical procedures

These are described in the preceding paper (Williams *et al.*, 1978) unless otherwise stated.

Detection of sugar phosphates in fractions after ion-exchange chromatography

Sugar phosphates were identified by treating 0.1ml samples taken from every second fraction with $Mg(NO_3)_2$ (Ames, 1966) and estimating total inorganic phosphate by the method of Taussky & Schorr (1953).

Isolation and purification of sugar phosphates from formate ion-exchange chromatography fractions

Fractions from formate ion-exchange chromatography (Bartlett & Bucolo, 1968) containing sugar

phosphates to be identified were pooled, the NH_4^+ removed by passage through a column of cation-exchange resin (Dowex 50W; X8; 50–100 mesh), the size of which was calculated from the approximate concentration of NH_4^+ and the exchange capacity of the resin. The column eluate together with 5 bed volumes of water washings were freeze-dried. The residue was dissolved in 0.2ml of water, applied as a discrete band to Whatman 3MM paper and chromatographed in solvent C. A small strip of the chromatogram (approx. 1cm wide) was selectively stained and a second strip of similar size was scanned for radioactivity.

Identification and analysis of heptulose 7-phosphate

The identification of *altro*-heptulose 7-phosphate (sedoheptulose 7-phosphate) and *manno*-heptulose 7-phosphate in purified heptulose 7-phosphate fractions (Fig. 1) involved the identification of the specific pentose 5-phosphate product formed by the transketolase-catalysed reaction (1).

$$\text{Heptulose-7-}P + \text{erythrose-4-}P \rightleftharpoons \text{fructose-6-}P + \text{pentose-5-}P \quad (1)$$

In reaction (1) *altro*-heptulose 7-phosphate and *manno*-heptulose 7-phosphate give rise to ribose 5-phosphate and arabinose 5-phosphate respectively. The reaction mixture contained in a volume of 3.0ml at 30°C: sample (0.1–5μmol); erythrose 4-phosphate (10μmol); $MgCl_2$ (15μmol); thiamin pyrophosphate (0.026μmol); transketolase (1 unit); glucose 6-phosphate dehydrogenase (2 units); glucose phosphate isomerase (2 units); $NADP^+$ (8μmol); glycylglycine/KOH buffer, pH7.4 (0.5mmol).

The progress and the rate of the reaction were monitored by observing the increase in A_{340}. When the reaction had reached equilibrium (approx. 40min) the entire mixture was passed through a small column (0.5cm × 10cm) of Dowex 50W (X8 resin; H^+ form), and the eluate together with 2vol. of water washings was concentrated to 0.2ml by evaporation under vacuum and then subjected to the procedure described below for the identification and quantification of ribose 5-phosphate and arabinose 5-phosphate.

Analysis and identification of D-*glycero*-D-*altro-octulose* 1,8-*bisphosphate and* D-*glycero*-D-*ido-octulose* 1,8-*bisphosphate*

The enzymic method used for the determination of D-*glycero*-D-*altro*-octulose 1,8-bisphosphate and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate involved the quantitative determination of dihydroxyacetone phosphate which resulted from an aldolase-catalysed cleavage (reaction 2).

$$\text{Octulose-1,8-}P_2 \rightleftharpoons \text{dihydroxyacetone-}P + \text{pentose-5-}P \quad (2)$$

The reaction mixture used was identical with that described for the determination of *altro*-heptulose 1,7-bisphosphate by Horecker (1965). Apart from the fact that the rate of cleavage of D-*glycero*-D-*ido*-octulose 1,8-bisphosphate was half that for D-*glycero*-D-*altro*-octulose 1,8-bisphosphate, this method did not discriminate between the two octulose bisphosphate isomers and could only be applied to samples free of fructose 1,6-bisphosphate, heptulose 1,7-bisphosphate and compounds that would yield dihydroxyacetone phosphate in the presence of aldolase.

The cysteine/H_2SO_4 method described by Bartlett & Bucolo (1968) was also used for the determination of octulose bisphosphate; the method was particularly useful for the analysis of octulose bisphosphate in fractions obtained from column chromatography.

The identification of D-*glycero*-D-*altro*-octulose 1,8-bisphosphate and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate in purified octulose bisphosphate fractions involved the identification of the product pentose 5-phosphate formed by the aldolase-catalysed reaction (2). In this reaction, D-*glycero*-D-*altro*-octulose 1,8-bisphosphate and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate give rise to ribose 5-phosphate and arabinose 5-phosphate respectively. The reaction mixture contained in a volume of 3.0ml at 30°C: sample (0.02–0.5μmol); aldolase (9 units); glycerol 3-phosphate dehydrogenase (2 units); NADH (1.0μmol); glycylglycine/KOH buffer, pH7.4 (0.5mmol). The progress and the rate of the reaction were monitored as described above. When the reaction had reached equilibrium (approx. 180min) the entire mixture was analysed for pentose phosphate content by the same method as was used for the identification of heptulose 7-phosphate isomers.

Analysis and identification of altro-heptulose 1,7-*bisphosphate*

The enzymic method used for the determination of *altro*-heptulose 1,7-bisphosphate was similar to that described for erythrose 4-phosphate (Williams *et al.*, 1978). When the reaction for the estimation of erythrose 4-phosphate had reached equilibrium, 5 units of aldolase was added. The subsequent increase in A_{340} was then used to calculate the concentration of *altro*-heptulose 1,7-bisphosphate; the method was found to be specific for this compound. *altro*-Heptulose 1,7-bisphosphate was also identified by paper chromatography and by the specific staining procedures of Klevstrand & Nordal (1950), and by its characteristic absorption spectrum after reaction with orcinol reagent (Horecker, 1957) (λ_{max} = 580nm).

Incorporation of ^{14}C into glucose 6-phosphate and fructose 6-phosphate

Methods for the isolation of glucose 6-phosphate and fructose 6-phosphate, and the determination of specific radioactivity were as described by Williams *et al.* (1971).

^{14}C-labelled volatile and acid-volatile compounds

The method used to detect any ^{14}C-labelled volatile compounds which may have been formed during the reaction of [U-^{14}C]ribose 5-phosphate with the rat liver enzyme preparation, involved measuring the radioactivity of 0.01 vol. of the aqueous distillate resulting from distillation of half the sample at pH 8.0 in a short-path distillation apparatus identical with that normally used for the distillation of ethanol (Vogel, 1967), the receiver flask being kept in an ice/water bath and the condenser maintained at 0°C by circulating ice-cold water through it. ^{14}C-labelled acid-volatile compounds were detected by measuring the radioactivity in 0.01 vol. of the aqueous distillate collected from the distillation of the same solution as above, but with the pH of the sample adjusted to 2.5.

Analysis and identification of ribose 5-phosphate and arabinose 5-phosphate

No specific enzymic or colorimetric method could be found in the literature for the estimation of arabinose 5-phosphate in a mixture of the other pentose phosphates and heptulose phosphates. Preliminary separation of the sugars therefore had to be achieved before the orcinol method of Mejbaum (1939) could be applied; this method is not specific for any one pentose ($\lambda_{max.}$ for both keto- and aldopentoses is 670nm). The following separation procedures were thus applied. Aqueous solutions containing pentose 5-phosphates and other sugar phosphates were concentrated to 0.2ml by evaporation under vacuum in a rotary evaporator, applied as a discrete band to Whatman 3MM paper and chromatographed in solvent system C. The resolution of pentose 5-phosphate from other sugar phosphates was complete and a pure fraction containing only pentose 5-phosphates was eluted from the chromatogram. The eluate was then analysed by either of two procedures. (i) It was treated with plant acid phosphatase (Williams *et al.*, 1971) and the free pentoses identified by paper chromatography in solvent systems A and B. From the pentose content (Mejbaum, 1939) and/or radioactivity content of the region of the chromatogram corresponding to authentic arabinose and ribose, the concentrations of arabinose and ribose and hence the 5-phosphate esters in the original sample were estimated. (ii) It was chromatographed in solvent system D. This solvent enabled arabinose 5-phosphate and ribose 5-phosphate to be separated from one another on the basis of different abilities to complex borate (borate only complexes with *cis*-hydroxy groups on adjacent carbon atoms). This solvent had the disadvantage that the R_F values of the pentose 5-phosphates were affected by the contaminating presence of low concentrations of inorganic ions.

Standard column profile and the preparation of reference sugar phosphates

D-*glycero*-D-*altro*-[^{14}C]*Octulose* 8-*phosphate*. This was prepared from [U-^{14}C]ribose 5-phosphate and fructose 6-phosphate in a transaldolase-catalysed reaction (3) (Racker & Schroeder, 1958).

Fructose-6-*P* + [U-^{14}C]ribose-5-*P* $\rightleftarrows$ D-*glycero*-D-*altro*-[4,5,6,7,8-^{14}C]octulose-8-*P* + glyceraldehyde-3-*P* (3)

The reaction mixture contained in a total volume of 8.0ml at 30°C: fructose 6-phosphate (10.0μmol); [U-^{14}C]ribose 5-phosphate (10.0μmol; 1.1×10^6 d.p.m.); transaldolase (2 units); triose phosphate isomerase (6 units); glycerol 3-phosphate dehydrogenase (3 units); NADH (15μmol); glycylglycine/KOH buffer, pH 7.4 (1mmol). The progress of the reaction and the rate of the formation of octulose 8-phosphate were determined by monitoring the decrease in A_{340} of a suitably diluted portion of the reaction mixture. After 180min when the reaction had reached equilibrium the entire reaction mixture was passed through a column (0.5cm × 5cm) of Dowex 50W (X8 resin; H^+ form; 200–400 mesh) to remove nicotinamide nucleotide and protein. The column eluate together with 2vol. of water washings was freeze-dried, dissolved in 0.2ml of water and chromatographed on Whatman 3MM paper in solvent C. The [^{14}C]octulose 8-phosphate was located by the specific staining method of Klevstrand & Nordal (1950) and by scanning the chromatogram for radioactivity. The solution of D-*glycero*-D-*altro*-[^{14}C]-octulose 8-phosphate (0.8mM) eluted from the paper chromatogram was stored as an aqueous preparation, pH 4.5, at −20°C.

D-*glycero* D-*altro*-[1,2,3-^{14}C]*Octulose* 1,8-*bisphosphate*. This was prepared from [U-^{14}C]dihydroxyacetone phosphate and ribose 5-phosphate in an aldolase-catalysed reaction (4):

[U-^{14}C]dihydroxyacetone-*P* + ribose-5-*P* $\rightleftharpoons$ D-*glycero*-D-*altro*-[1,2,3-^{14}C]octulose-1,8-P_2 (4)

The reaction mixture was contained in a total volume of 6.0ml at 30°C: [U-^{14}C]dihydroxyacetone phosphate, prepared from [U-^{14}C]glycerol as described by Clark *et al.* (1972) (15μmol; 1×10^7 d.p.m.); ribose 5-phosphate (15μmol); triethanolamine hydrochloride buffer, pH 7.6 (1.6mmol); crystalline

rabbit muscle aldolase (9 units). After 3h when the yield of octulose bisphosphate was approx. 5μmol, the entire reaction mixture was treated as described above for the isolation of octulose 8-phosphate. The [^{14}C]octulose bisphosphate was located by the specific staining technique (Klevstrand & Nordal, 1950), which was coincident with a peak of radioactivity. The solution of octulose bisphosphate (2.5mM) eluted from the paper chromatogram was stored as an aqueous preparation at pH4.5 and at −20°C. Unlabelled D-*glycero*-D-*altro*-octulose 1,8-bisphosphate was prepared by the same method, except that unlabelled dihydroxyacetone phosphate (15μmol) replaced the [U-^{14}C]dihydroxyacetone phosphate.

altro-Heptulose 1,7-*bisphosphate*. This was prepared and isolated in a similar manner to that described for D-*glycero*-D-*altro*-[^{14}C]octulose 1,8-bisphosphate, except that erythrose 4-phosphate (15μmol) replaced ribose 5-phosphate in the preparation and unlabelled dihydroxyacetone phosphate (15μmol) was used (reaction 5).

$$\text{Dihydroxyacetone-}P + \text{erythrose-4-}P \rightleftarrows \textit{altro}\text{-heptulose-1,7-}P_2 \quad (5)$$

Under the conditions specified, the yield of *altro*-heptulose 1,7-bisphosphate was 80–85% in 30min.

D-*glycero*-D-*ido-Octulose* 1,8-*bisphosphate*. This was prepared and isolated in a manner identical with that already described for D-*glycero*-D-*altro*-[^{14}C]-octulose 1,8-bisphosphate, except that arabinose 5-phosphate replaced ribose 5-phosphate and dihydroxyacetone phosphate replaced [U-^{14}C]dihydroxyacetone phosphate in reaction (4).

Kinetics of transaldolase inhibition by arabinose 5-phosphate

The initial velocity of the transaldolase-catalysed reaction (6) in the forward direction was determined by recording the rate of decrease in A_{340} due to the oxidation of NADH in the following assay system. Each cuvette was preincubated at 30°C for 10min, and contained in a total volume of 1.0ml: glycylglycine/KOH buffer, pH7.4 (0.125mmol); NADH (0.2μmol); triose phosphate isomerase (0.5 unit); glycerol 3-phosphate dehydrogenase (0.5 unit); fructose 6-phosphate (5μmol). Five concentrations of erythrose 4-phosphate were used (250, 150, 33, 15.3 and 10μM) together with three concentrations of the inhibitor arabinose 5-phosphate (0.2, 0.3 and 0.4mM). The reaction was commenced by the addition of 5μl of crystalline transaldolase (approx. 0.05 unit). The initial velocity of reaction (6) in the reverse direction was determined by recording the increase in A_{340} due to the reduction of $NADP^+$ in the following assay system. Each cuvette was preincubated, as above, and contained in a total volume of 1.0ml: glycylglycine/KOH buffer, pH7.4 (0.125 mmol); $NADP^+$ (0.5μmol); glucose phosphate isomerase (0.5 unit); glucose 6-phosphate dehydrogenase (0.5 unit) and *altro*-heptulose 7-phosphate (5.0μmol). Five concentrations of D-glyceraldehyde 3-phosphate were used (2.5, 1.5, 0.33, 0.153 and 0.1mM) together with three concentrations of the inhibitor arabinose 5-phosphate (as above), the reaction being commenced by the addition of 5μl of crystalline transaldolase (approx. 0.05 unit):

$$\text{Fructose-6-}P + \text{erythrose-4-}P \rightleftharpoons \textit{altro}\text{-heptulose-7-}P + \text{glyceraldehyde-3-}P \quad (6)$$

Results

Possible new reactions of the pentose phosphate pathway involving volatile and non-phosphorylated intermediates

Table 1 shows the results of experiments designed to detect reactions not previously attributed to the non-oxidative pentose phosphate pathway, which, considered individually or in combination with other known reactions, could theoretically account for the anomalous distribution of ^{14}C in glucose 6-phosphate formed from [1-^{14}C]ribose 5-phosphate at short incubation time intervals [see Table 5 of the preceding paper (Williams *et al.*, 1978)]. No reactions involving volatile products were detected (Table 1).

Identification of new metabolite intermediates in the enzyme-catalysed reactions synthesizing hexose 6-phosphate from ribose 5-phosphate

A proportion of substrate carbon (a maximum of 19.2% at 30min) could not be accounted for when all the intermediates currently assigned to the non-oxidative pentose phosphate pathway had been determined during a repeat of the Horecker *et al.* (1954) experiment (Table 4 of Williams *et al.*, 1978). Since free P_i did not accumulate during the incubation (Horecker *et al.*, 1954; Williams *et al.*, 1978), attention was focused on phosphorylated sugars in the search for new intermediates of pentose 5-phosphate metabolism which would lead to the satisfactory accounting of all of the carbon in the reaction mixtures. This search was also made to find and isolate the intermediates whose nature and isotope labelling patterns would provide an explanation of the C-2, C-6 labelling in hexose 6-phosphate both *in vitro* (Williams *et al.*, 1978) and *in situ* (Williams *et al.*, 1971).

The chromatographic separation of sugar phosphates was achieved by the method of Bartlett & Bucolo (1968). A reference column profile (Fig. 1*a*) was obtained with the following authentic sugar phosphates: D-*glycero*-D-*altro*-[^{14}C]octulose 8-phos-

Table 1. *Examination of a selection of ^{14}C-labelled substrates which theoretically may be expected to react with liver enzymes and give rise to 2,6-^{14}C-labelling patterns in hexose 6-phosphates*

Each reaction mixture was contained in a volume of 3.0ml at 25°C: 3.0 mg of the rat liver enzyme preparation (RLEP) (Williams *et al.*, 1978); glycylglycine/KOH buffer, pH7.4 (0.5mmol); substrate(s) as indicated (5μCi of each radioactive compound was used). Samples (0.5ml) were removed at 0, 5 and 30min and at 3, 8 and 17h for analysis. The procedures used to detect volatile and acid-volatile compounds are described in the Experimental section. The test of the incorporation of ^{14}C from the ^{14}C-labelled substrates into hexose 6-phosphates was made by measuring the radioactivity in glucose 6-phosphate and fructose 6-phosphate isolated from the samples (Williams *et al.*, 1971) taken from the reaction mixtures at the specified intervals.

Experiments designed to test for:	Result
Release of $^{14}CO_2$	
RLEP + 10μmol of [U-^{14}C]ribose-5-*P*	−
+ 10μmol of [U-^{14}C]ribose-5-*P* + 5mol of $NADP^+$ (1)*	+
Formation of volatile radioactive compounds	
RLEP + 10μmol of [U-^{14}C]ribose-5-*P*	−
Formation of acid-volatile radioactive compounds	
RLEP + 10μmol of [U-^{14}C]ribose-5-*P*	−
Incorporation of ^{14}C into hexose-6-*P*	
RLEP + 10μmol of ribose-5-*P* + 5μmol of $NaH^{14}CO_3$	−
+ 10μmol of ribose-5-*P* + 5μmol of $NaH^{14}CO_3$ + 2μmol of NAD^+	−
+ 10μmol of ribose-5-*P* + 5μmol of $NaH^{14}CO_3$ + 2μmol of NADH	−
+ 10μmol of ribose-5-*P* + 5μmol of $NaH^{14}CO_3$ + 2μmol of $NADP^+$	−
+ 10μmol of ribose-5-*P* + 5μmol of $NaH^{14}CO_3$ + 2μmol of NADPH	−
Ribulose bisphosphate carboxylase (18μg) + 5μmol of $NaH^{14}CO_3$ + 5μmol of ribulose-1,5-P_2 (2)	+
RLEP + 10μmol of ribose-5-*P* + 1μmol of [^{14}C]formaldehyde	−
+ 10μmol of ribose-5-*P* + 1μmol of sodium [^{14}C]formate	−
+ 10μmol of ribose-5-*P* + 1μmol of [U-^{14}C]glycerol	−
+ 10μmol of ribose-5-*P* + 1μmol of sodium [U-^{14}C]pyruvate	−
+ 10μmol of ribose-5-*P* + 0.5μmol of sodium [U-^{14}C]acetate	−
+ 10μmol of ribose-5-*P* + 0.1μmol of [^{14}C]methanol	−
+ 10μmol of ribose-5-*P* + 2μmol of [U-^{14}C]glucose	−
+ 10μmol of ribose-5-*P* + 2μmol of [U-^{14}C]ribose	−
+ 10μmol of ribose-5-*P* + 0.5μmol of sodium [U-^{14}C]glycollate	−
+ 10μmol of ribose-5-*P* + 0.5μmol of sodium [U-^{14}C]glyoxalate	−
+ 10μmol of ribose-5-*P* + 1μmol of [U-^{14}C]erythrose-4-*P* (3)	+

* Reactions (1), (2) and (3) were included to check the efficacy of the test systems.

phate, *altro*-heptulose 7-phosphate, fructose 6-phosphate, ribose 5-phosphate, DL-glyceraldehyde 3-phosphate, D-*glycero*-D-*altro*-[^{14}C]octulose 1,8-bisphosphate and *altro*-heptulose 1,7-bisphosphate. The column profile (Fig. 1*a*) is consistent with that obtained under similar conditions by Bartlett & Bucolo (1968) (see their Fig. 1). In Figs. 1(*b*), 1(*c*), 1(*d*), 1(*e*) and 1(*f*) results are shown for the chromatographic analysis of the incubation mixtures, composed of the rat liver enzyme preparation and [U-^{14}C]-ribose 5-phosphate sampled at 0, 0.5, 3, 12 and 17h respectively. Five additional intermediates of carbohydrate metabolism in mammalian liver were detected and identified in the fractions from the column profiles.

Samples of the incubation reaction mixture removed at 0.5h and at all later intervals contained significant amounts of ^{14}C in column-chromatography fractions corresponding to the regions of the chromatograms eluted with 1.45M-ammonium formate (Figs. 1*c*, 1*d*, 1*e* and 1*f*) and coincident with the position occupied by authentic D-*glycero*-D-*ido*-octulose 1,8-bisphosphate (Fig. 1*a*). Analysis of the appropriately pooled fractions at each time interval confirmed the presence of octulose 1,8-bisphosphate (it satisfied the criteria of data in Table 2) and showed that the amount of this intermediate increased to a maximum of 3.1μmol (0.313mM) at 17h. This value corresponded to 4.6% of the total substrate carbon. The properties of the octulose 1,8-bisphosphate isolated by ion-exchange column chromatography of the 0.5h and all later samples (Figs. 1*c*, 1*d*, 1*e* and 1*f*) are shown in Table 2 together with the properties of three isomers of octulose phosphate. An analysis

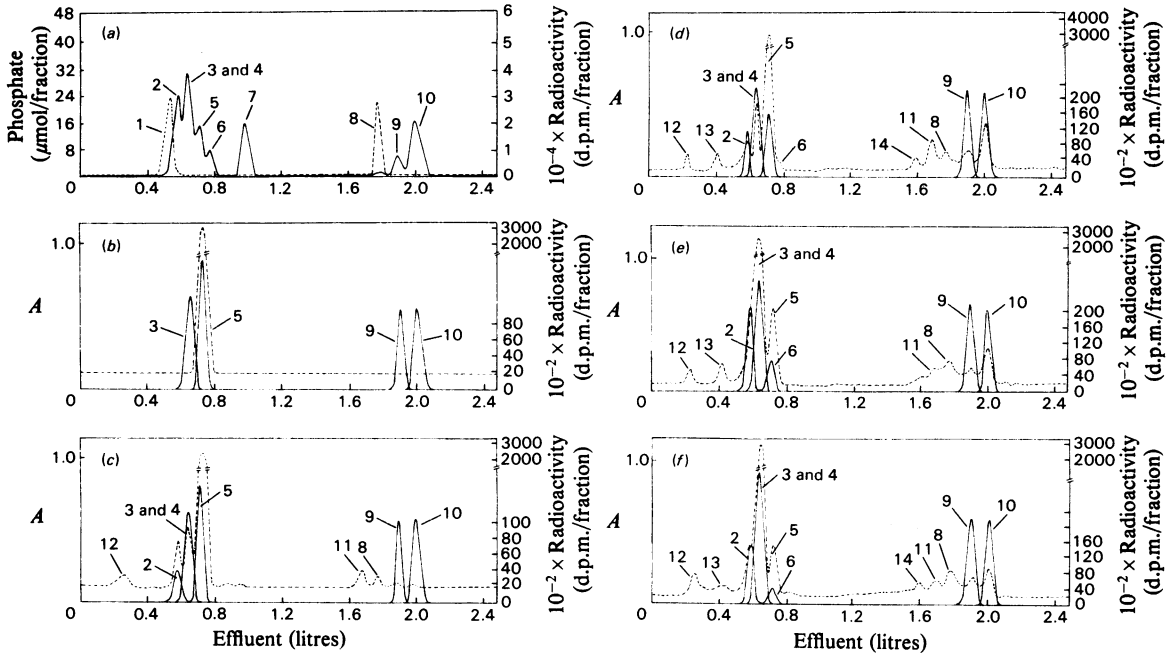
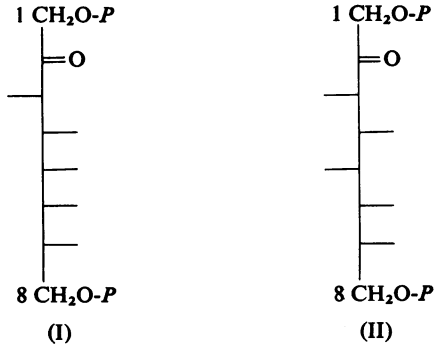


Fig. 1. *Chromatographic analysis of the products of [U-^{14}C]ribose 5-phosphate metabolism by rat liver enzymes*

The incubation mixture contained in a total volume of 35.0ml at 25°C: [U-^{14}C]ribose 5-phosphate (360μmol and 4×10^7 d.p.m.) and 2.5ml of the rat liver enzyme preparation (73.0mg of protein). Samples of 6.0ml were removed from the incubation at 0, 0.5, 3, 12 and 17h and the protein was removed (see the Experimental section). The samples were then subjected to the ion-exchange chromatographic procedure of Bartlett & Bucolo (1968). To check the ability of this system to resolve sugar phosphates, a mixture of authentic sugar phosphates was first chromatographed (*a*). The following sugar phosphates were dissolved in 25ml of water, the pH adjusted to 4.5 and the solution together with 50ml of water applied to a column (1.5cm × 30cm) of Dowex 1 (X8 resin; formate form): D-*glycero*-D-*altro*-[^{14}C]octulose 8-phosphate (2μmol and 10^5 c.p.m.) (1); *altro*-heptulose 7-phosphate (50μmol) (2); fructose 6-phosphate (50μmol) (3); glucose 6-phosphate (50μmol) (4); ribose 5-phosphate (50μmol) (5); DL-glyceraldehyde 3-phosphate (25μmol) (6); sodium orthophosphate (50μmol) (7); D-*glycero*-D-*altro*-[^{14}C]octulose 1,8-bisphosphate (3.5μmol and 10^5 c.p.m.) (8); *altro*-heptulose 1,7-bisphosphate (10μmol) (9); fructose 1,6-bisphosphate (25μmol) (10). Sugar phosphates were eluted from the column with a linear gradient of 6 litres of 0–5M-ammonium formate (acid/salt, 4:1, v/v) as described by Bartlett & Bucolo (1968). Every second fraction (0.1ml) was monitored for radioactivity (----) and total phosphate (——) (see Experimental section). (*b*) Chromatographic analysis of the sample removed from the incubation at 0min. Authentic fructose 6-phosphate (50μmol) (3), fructose 1,6-bisphosphate (50μmol) (10) and *altro*-heptulose 1,7-bisphosphate (50μmol) (9) were each added and the sample was applied to a column of Dowex 1(X8; formate resin) as described for (*a*). The positions of the authentic sugar phosphates were determined by colorimetric methods (——) of every second fraction. A 0.5ml portion was used for the detection of the hexose phosphates ($\lambda_{max.} = 625$nm) by the method of Graham & Smydzuk (1965), and 0.5ml for the detection of the substrate ribose 5-phosphate ($\lambda_{max.} = 670$nm) and heptulose phosphate ($\lambda_{max.} = 580$nm) (Mejbaum, 1939). Every second fraction was monitored for radioactivity, using a 0.1ml sample (----). (*c*) Chromatographic analysis of the sample removed from the incubation reaction mixture at 30min, experimental details as for (*b*). Methods for identification of individual sugar phosphates are given in the Experimental section. Peak (12) was identified as dihydroxyacetone and peak (11) was not identified. (*d*) Chromatographic analysis of the 3h sample. Peak (13) was identified as glucose 1-phosphate; peak (14) was unidentified. (*e*) and (*f*) Results of the chromatographic examination of the samples removed at 12 and 17h respectively. Background radioactivity (approx. 18 c.p.m.) was not subtracted from the radioactivity measurements on column fractions. Radioactivity and total phosphate analysis indicated that 98% of the total material applied to the column had been eluted by collection of 220 × 11.5ml fractions at a constant flow rate of 45ml/h.

of the aldolase cleavage products (see the Experimental section) of the isolated octulose 1,8-bisphosphate fraction [reaction (2) and Table 2] showed that there were two octulose bisphosphate isomers present, since both ribose 5-phosphate and arabinose 5-phosphate were identified as the pentose 5-phosphate components; thus the two isomers of octulose 1,8-bisphosphate were concluded to be D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (I) and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate (II).

Analysis of the octulose 1,8-bisphosphate, isolated at various times throughout the 17h incubation, showed that the composition varied only slightly and that it was composed of approx. 88 and 12% D-*glycero*-D-*altro*-octulose 1,8-bisphosphate and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate respectively.



(I) (II)

altro-Heptulose 1,7-bisphosphate was isolated and identified in relatively small quantities from fractions corresponding to the position occupied by authentic *altro*-heptulose 1,7-bisphosphate. The concentration of *altro*-heptulose 1,7-bisphosphate increased throughout the incubation to a maximum at 17h, and the values obtained for the concentration of this compound, by radiochemical analysis after its separation by liquid (Figs. 1*c*, 1*d*, 1*e* and 1*f*) and paper chromatography, agreed with values determined by enzymic analysis (Table 3).

Fractions containing pentose 5-phosphate after column chromatography (Figs. 1*c*, 1*d*, 1*e* and 1*f*) were examined for the presence of arabinose 5-phosphate, since arabinose 5-phosphate was identified as one of the aldolase cleavage products of the octulose 1,8-bisphosphate isolated from the same reaction mixture (see above).

The pentose 5-phosphate fraction was purified by paper chromatography in solvent system C. The resultant pure pentose 5-phosphates were dephosphorylated, and the composition of the pentose

Table 2. *Properties of octulose* 1,8-*bisphosphate formed from ribose* 5-*phosphate in reactions catalysed by rat liver enzyme preparation*

Fractions containing octulose 1,8-bisphosphate isolated from the formate ion-exchange column chromatography of the 30min, 3, 12 and 17h samples (Figs. 1*c*, 1*d*, 1*e* and 1*f*) were individually pooled and ammonium formate was removed (see the Experimental section). The properties of the individually isolated and purified octulose 1,8-bisphosphates were compared with properties of authentic D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and D-*glycero*-D-*altro*-octulose 1,8-bisphosphate prepared as described in the Experimental section. For comparison some properties of L-*glycero*-L-*galacto*-octulose 1-phosphate are included (Jones & Sephton, 1960). Methods for the determination of the rates of aldolase-catalysed cleavage of octulose 1,8-bisphosphate and for the identification of cleavage products are given in the Experimental section.

	Isomer of octulose phosphate			
	D-*glycero*-D-*ido*-Octulose 1,8-bisphosphate	D-*glycero*-D-*altro*-Octulose 1,8-bisphosphate	L-*glycero*-L-*galacto*-Octulose 1-phosphate	Isolated octulose 1,8-bisphosphate
Molarity of ammonium carbonate required for elution from formate column (Bartlett & Bucolo, 1968)	1.45	1.45	—	1.45
R_{PO_4} in solvent C	0.12	0.12	—	0.12
$\lambda_{max.}$ (nm) with cysteine/H_2SO_4 reagent	410	485	485	410
A_{410}/A_{485} (Bartlett & Bucolo, 1960)	10.5	0.82*		1.6†
Rate of aldolase cleavage at 30°C (relative to the *altro* isomer)	0.59	1.0	—	0.69
Products of aldolase cleavage	Dihydroxyacetone-*P*/arabinose-5-*P* 1:1	Dihydroxyacetone-*P*/ribose-5-*P* 1:1	Dihydroxyacetone-*P*/L-arabinose 1:1	Dihydroxyacetone-*P*/ribose-5-*P*/arabinose-5-*P* 12.5:11.4:1.0
Phosphate/carbohydrate ratio	2:1	2:1	1:1	2:1
R_F of octulose in solvent A after dephosphorylation	0.17 0.41 (anhydro)	0.17	0.17	0.17

* Bartlett & Bucolo (1960) report $\lambda_{max.} = 485$nm and $A_{410}/A_{485} = 0.5$.

† Values obtained for 30min time sample.

mixture was resolved in solvent systems A and B; xylulose, arabinose and ribose were identified, thus it was concluded that these pentoses were derived from xylulose 5-phosphate, arabinose 5-phosphate and ribose 5-phosphate respectively. The concentration of arabinose 5-phosphate increased to a maximum at 0.5h and then slowly decreased during the remaining period of the incubation (Table 3).

The fractions corresponding to *altro*-heptulose 7-phosphate were analysed for the presence of *manno*-heptulose 7-phosphate, since arabinose 5-phosphate was identified in the incubation reaction mixture and it is a known aldehyde acceptor in a transketolase-catalysed reaction (Datta & Racker, 1961), reaction (7).

Arabinose-5-*P* + ketol donor ⇄ *manno*-heptulose-7-*P* + aldehyde acceptor (7)

An examination of the reaction products formed from the transketolase-catalysed assay of heptulose 7-phosphate [Experimental section, reaction(1)], isolated from the appropriate fraction after ion-exchange chromatography (Figs. 1*c*, 1*d*, 1*e* and 1*f*), was made to establish the identity of the isomer(s) of heptulose 7-phosphate formed during the incubation of [U-^{14}C] ribose 5-phosphate with the rat liver enzyme preparation. Paper-chromatographic analysis (solvent systems A and B) of the dephosphorylated pentose 5-phosphate reaction products (after their prior chromatographic purification in solvent system C) revealed the presence of two pentoses, ribose and arabinose, thus showing that *altro*-heptulose 7-phosphate (III) and *manno*-heptulose 7-phosphate (IV) were present in all the fractions of heptulose 7-phosphate isolated at 0.5h and all sampling intervals thereafter. Chromatographic analysis of the purified pentose 5-phosphate mixture in solvent system D also confirmed the presence of these two isomers of heptulose 7-phosphate.

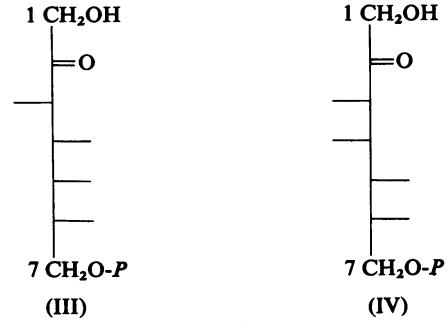


Table 3. *Composition of reaction mixture and carbon balance for reactions forming hexose 6-phosphate from ribose 5-phosphate catalysed by rat liver enzyme preparation*

The data of Williams *et al.* (1978) (their Table 4) have been extended to include results for the determination of the concentrations of each of the new intermediates (Fig. 1). Methods for the determination of the new intermediates at the times shown are given in the Experimental section. Each value is expressed as a percentage of the total carbon contributed by the substrate ribose 5-phosphate at the commencement of the incubation.

	Composition				
Metabolite \ Incubation time (h) ...	0	0.5	3	12	17
Ribose-5-*P*	100	55.0	22.9	9.3	7.5
Xylulose-5-*P*	0	13.6	5.6	0.8	0
Ribulose-5-*P*	0	0	0	0	0
Arabinose-5-*P*	0	16.2	5.2	1.2	1.0
D-*altro*-Heptulose-7-*P*	0	4.0	23.1	26.1	18.3
D-*manno*-Heptulose-7-*P*	0	3.4	10.0		
Glyceraldehyde-3-*P*	0	0.2	0	0	0
Dihydroxyacetone-*P*	0	1.3	5.3	4.0	3.0
Fructose-1,6-P_2	0	1.7	12.0	8.3	7.4
D-*altro*-Heptulose-1,7-P_2	0	0.2	1.7	2.1	2.3
D-*glycero*-D-*altro*-Octulose-1,8-P_2	0	1.1	2.8	3.5	4.1
D-*glycero*-D-*ido*-Octulose-1,8-P_2	0	0.1	0.4	0.5	0.5
Erythrose-4-*P*	0	0	0	0	0
Glucose-1-*P*	0	0.1	0.3	1.1	1.8
Fructose-6-*P*	0	0.5	1.8	11.0	16.0
Glucose-6-*P*	0	1.3	6.0	29.2	36.2
Total	100	98.7	97.1	97.1	98.1
Percentage of original carbon of ribose 5-phosphate not accounted for	0	1.3	2.9	2.9	1.9

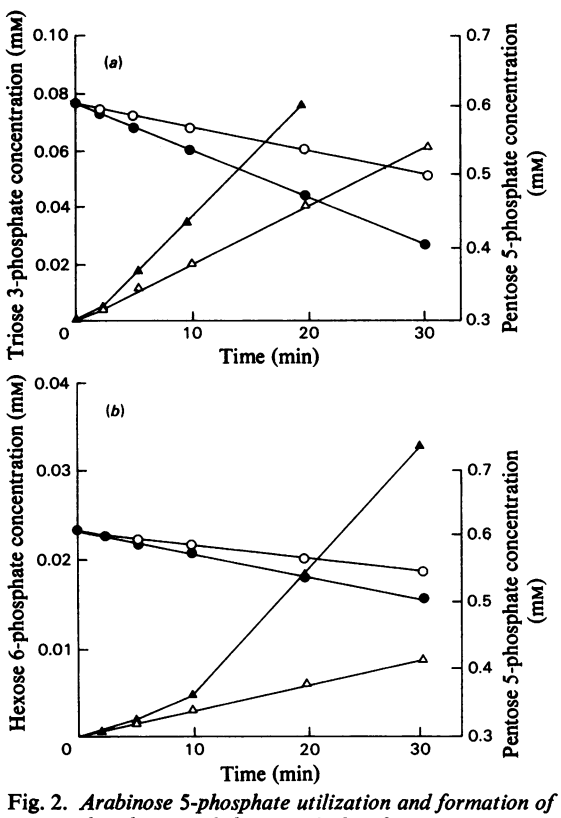


Fig. 2. *Arabinose 5-phosphate utilization and formation of triose phosphate and hexose 6-phosphate in reactions catalysed by the rat liver enzyme preparation*

(*a*) Each reaction mixture in a total volume of 3.3ml at 25°C contained: pentose 5-phosphate (2.0μmol of arabinose 5-phosphate or ribose 5-phosphate); 0.05ml of rat liver enzyme (1.46mg of protein); glycylglycine/KOH buffer, pH7.4 (750μmol); NADH, 1.0μmol; triose phosphate isomerase (0.2 unit); glycerol 3-phosphate dehydrogenase (0.2 unit). The formation of triose phosphate from arabinose 5-phosphate (△) and from ribose 5-phosphate (▲) was measured by monitoring the decrease in A_{340} due to the oxidation of NADH, when read against a control cuvette containing all components except pentose 5-phosphate. Utilization of arabinose 5-phosphate (○) and ribose 5-phosphate (●) was determined (Mejbaum, 1939) on samples of 0.3ml removed from test and control incubations. The results are means of duplicate determinations from three experiments. (*b*) Each reaction mixture in a total volume of 3.3ml at 25°C contained: pentose 5-phosphate (2.0μmol of arabinose 5-phosphate or ribose 5-phosphate); 0.05ml of rat liver enzyme (1.46mg of protein); glycylglycine/KOH buffer, pH7.4 (750μmol); $NADP^+$ (1.0μmol); glucose phosphate isomerase (0.2 unit); glucose 6-phosphate dehydrogenase (0.2 unit). Formation of hexose 6-phosphate from arabinose 5-phosphate (△) and from ribose 5-phosphate (▲) was measured by monitoring the increase in A_{340} due to the reduction of $NADP^+$ (control cuvette contained all components except pentose 5-phosphate). Utilization of arabinose 5-phosphate (○) and ribose 5-phosphate (●) was determined as described for (*a*).

Carbon-balance studies of intermediates involved in the formation of hexose 6-phosphate from ribose 5-phosphate in reactions catalysed by the rat liver enzyme preparation

Results of the ion-exchange chromatography of the products of [U-^{14}C]ribose 5-phosphate metabolism by the rat liver enzyme preparation (Fig. 1) and the data of Williams *et al.* (1978) (see their Table 4) were compiled in an attempt to obtain a more complete carbon balance for the rat liver enzyme-catalysed synthesis of hexose 6-phosphate from ribose 5-phosphate. These results are shown in Table 3 and at all times examined, over 97% of the substrate carbon was accounted for.

Conversion of arabinose 5-phosphate to triose 3-phosphate and hexose 6-phosphate in reactions catalysed by the rat liver enzyme preparation

The rate of arabinose 5-phosphate disappearance from the reaction mixture together with the rate of formation of triose 3-phosphate are shown in Fig. 2(*a*). For the purpose of comparison an identical incubation was conducted which contained ribose 5-phosphate (a known metabolite of liver metabolism) in place of arabinose 5-phosphate. The maximum rate of disappearance of arabinose 5-phosphate was 7.56nmol/min per mg of protein which was approximately half the rate obtained for the utilization of ribose 5-phosphate.

The rates of arabinose 5-phosphate utilization and hexose 6-phosphate formation are shown in Fig. 2(*b*). For comparison, an identical incubation was conducted which contained ribose 5-phosphate instead of arabinose 5-phosphate. The rate of arabinose 5-phosphate utilization was 3.75nmol/min per mg of protein, which was approximately half the rate of ribose 5-phosphate utilization. The rate of production of hexose 6-phosphate (fructose 6-phosphate and glucose 6-phosphate determined enzymically) differed markedly for the two pentose 5-phosphate substrates. For ribose 5-phosphate there was an initial lag phase of approx. 10min during which time only a relatively slow production of hexose 6-phosphate occurred. After the lag phase there was an increased rate which reached a maximum linear value of 3.05nmol/min per mg of protein. This increased rate was approximately 5-fold greater than the maximum linear rate of hexose 6-phosphate formation from arabinose 5-phosphate.

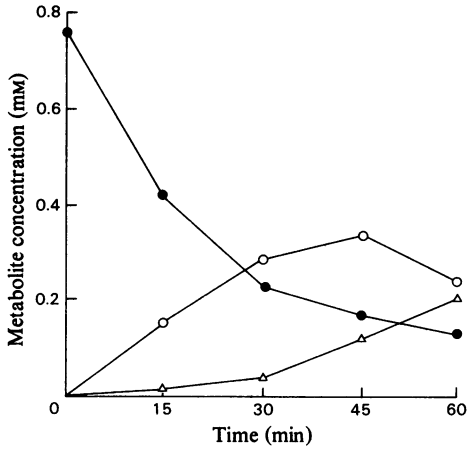


Fig. 3. *Interconversion of arabinose 5-phosphate with other pentose 5-phosphates in reactions catalysed by rat liver enzyme preparation during the first hour of incubation*

The incubation contained in a total volume of 33.0ml at 25°C: arabinose 5-phosphate (25.0μmol); rat liver enzyme preparation (2.5ml; 73.0mg of protein); glycylglycine/KOH buffer, pH7.4 (7.0mmol). Samples of 5.0ml were removed, treated to remove protein (see Experimental section) and the volumes adjusted to 10ml. A sample (1.0ml) was removed for enzymic analysis of ribose 5-phosphate (○), ribulose 5-phosphate and xylulose 5-phosphate (△) (Williams *et al.*, 1978) and 8.0ml for chromatographic analysis of free sugars using solvent system A, following dephosphorylation (Williams *et al.*, 1971). The concentration of arabinose 5-phosphate (●) was determined as detailed in the Experimental section.

Interconversion of arabinose 5-phosphate into other pentose 5-phosphates and heptulose 7-phosphate

Fig. 3 shows the results of experiments in which arabinose 5-phosphate was incubated with the rat liver enzyme preparation, and the resulting pentose 5-phosphates were assayed. A paper-chromatographic examination of the reaction products during the first 1h of incubation confirmed the enzymic analysis of the pentose phosphate, namely that the first pentose 5-phosphate to be formed from arabinose 5-phosphate was ribose 5-phosphate. Heptulose was only able to be detected on chromatograms after 45min.

Utilization of arabinose 5-phosphate in reactions catalysed by enzymes of liver and other rat tissues

The rat liver enzyme preparation utilized arabinose 5-phosphate to produce both triose 3-phosphate (Fig. 2*a*) and hexose 6-phosphate (Fig. 2*b*). As ribose 5-phosphate reactions resulted in the formation of hexose 6-phosphate when incubated with enzyme preparations prepared from tissues from a wide variety of sources (Glock & McLean, 1954), experiments were conducted with enzyme preparations from a variety of rat tissues to ascertain whether arabinose 5-phosphate was similarly metabolized.

A comparison of the relative rates of arabinose 5-phosphate and ribose 5-phosphate utilization for various rat tissues is shown in Table 4. The enzyme preparation used in each experiment was substrate- and coenzyme-free and was prepared by extraction of an acetone-dried powder of the respective tissue in 12 vol. of 0.25M-glycylglycine/KOH buffer, pH7.4 (Williams *et al.*, 1978). Substrate-free diffusates of tissue homogenates were also used occasionally and gave similar results; however, the acetone-dried powders of tissues were preferred, since they could be stored, desiccated at 0–4.0°C, for several weeks without any appreciable loss of enzyme activity occurring.

For liver, the rate of arabinose 5-phosphate utilization was approx. 0.33-fold that for ribose 5-phosphate. Most tissues, except for liver, brain cortex, brain medulla and skeletal muscle, showed rates of arabinose 5-phosphate utilization slightly less than that for ribose 5-phosphate and in all cases arabinose 5-phosphate utilization never exceeded that of ribose 5-phosphate. The results in Table 4 are similar to those obtained by Glock & McLean (1954) for the rate of ribose 5-phosphate breakdown in supernatants of dialysed tissue.

Table 4 also shows that hexose 6-phosphate was produced from arabinose 5-phosphate and ribose 5-phosphate by enzyme preparations from the various tissues of the rat. For liver, arabinose 5-phosphate gave a rate of hexose 6-phosphate production approx. 0.25-fold that observed for ribose 5-phosphate, and for heart, skeletal muscle, pancreas and thryoid similar rates were obtained for both pentose 5-phosphates.

The rate of production of triose 3-phosphate from arabinose 5-phosphate and ribose 5-phosphate for the various rat tissues was measured by the method described in the legend of Fig. 2(*a*). For liver the rate of triose 3-phosphate production from arabinose 5-phosphate was 0.45-fold that of the rate of its formation from ribose 5-phosphate. For heart, spleen, pancreas and thyroid, arabinose 5-phosphate formed triose 3-phosphate at 0.91–0.98 times the rate of formation from ribose 5-phosphate. In the coupled enzyme system used to measure the rate of triose 3-phosphate formation by the rat liver enzyme preparation (Table 4), the co-product produced with triose 3-phosphate after metabolism of arabinose 5-phosphate was identified as a heptulose 7-phosphate by the colorimetric method of Mejbaum (1939) and by a paper-chromatographic examination of the reaction products in solvent C.

Table 4. *Relative rates of utilization of arabinose 5-phosphate and ribose 5-phosphate in reactions catalysed by enzyme preparations from various rat tissues*

Each incubation mixture contained in a volume of 3.0ml at 25°C: pentose 5-phosphate (2.0μmol of either arabinose 5-phosphate or ribose 5-phosphate); enzyme preparation (2.5mg of protein), see the Experimental section; glycylglycine/KOH buffer, pH7.4 (0.5mmol). For the measurement of the rate of utilization of pentose 5-phosphate, samples of 0.5ml were removed from the incubation and deproteinized at 0, 5 and 10min, and from a control incubation, which did not contain pentose 5-phosphate. The supernatant was analysed for pentose 5-phosphate content by the procedure described in the legend of Fig. 2. Rates of hexose 6-phosphate production were determined by enzymic analysis for glucose 6-phosphate and fructose 6-phosphate (Hohorst, 1965) in deproteinized samples removed at 0, 30 and 60min from each incubation mixture described above. The initial rate of triose phosphate formation was determined in the coupled assay system described in the legend to Fig. 2. Each result is the mean of duplicate determinations from two experiments. Units are expressed as nmol/min per mg of protein.

	Utilization of pentose 5-phosphate		Production of hexose 6-phosphate		Production of triose phosphate	
	Arabinose 5-phosphate	Ribose 5-phosphate	Arabinose 5-phosphate	Ribose 5-phosphate	Arabinose 5-phosphate	Ribose 5-phosphate
Liver	1.09	3.65	0.06	0.26	1.31	2.90
Brain cortex	0.08	2.75	0.02	0.21	0.06	0.18
Brain medulla	0.38	0.73	0.01	0.18	0.04	0.24
Heart	0.16	0.17	0.04	0.04	0.14	0.15
Spleen	1.29	1.44	0.14	0.32	0.24	0.27
Kidney	1.41	1.48	0.01	0.05	0.37	1.63
Skeletal muscle	1.82	3.03	0.10	0.11	0.20	0.29
Testis	2.14	2.22	0.02	0.12	0.44	0.64
Pancreas	0.76	0.83	0.03	0.03	0.14	0.15
Thyroid	2.00	2.54	0.10	0.14	0.21	0.22

Table 5. *Rate of production of hexose 6-phosphate from octulose bisphosphate in reactions catalysed by the rat liver enzyme preparation*

The reaction mixtures in a volume of 3.3ml contained: 1.0μmol of each substrate; 0.05ml of the rat liver enzyme preparation (1.46mg of protein); glycylglycine/KOH buffer, pH7.4 (0.75mmol); 1.0μmol of $NADP^+$; 0.2 unit of glucose phosphate isomerase and 0.2 unit of glucose 6-phosphate dehydrogenase. The rate of hexose 6-phosphate formation was measured by monitoring the increase in A_{340} against a control cuvette which contained all components except substrate. The results shown are mean values from three experiments.

Substrate	Rate of hexose 6-phosphate formation (nmol/min per mg of protein)
None	0.0
D-*glycero*-D-*ido*-Octulose-1,8-P_2	0.29
D-*glycero*-D-*altro*-Octulose-1,8-P_2	0.26
Fructose-1,6-P_2	0.0
Erythrose-4-*P*	0.0
Glyceraldehyde-3-*P*	0.0
D-*glycero*-D-*ido*-Octulose-1,8-P_2 + erythrose-4-*P*	2.41
D-*glycero*-D-*altro*-Octulose-1,8-P_2 + erythrose-4-*P*	0.45
D-*glycero*-D-*ido*-Octulose-1,8-P_2 + glyceraldehyde-3-*P*	0.28
D-*glycero*-D-*altro*-Octulose-1,8-P_2 + glyceraldehyde-3-*P*	0.17
D-*glycero*-D-*ido*-Octulose-1,8-P_2 + erythrose-4-*P* (with no rat liver enzyme preparation)	0.0
Fructose-1,6-P_2 + erythrose-4-*P*	0.0
altro-Heptulose-7-*P*	0.02
D-*glycero*-D-*ido*-Octulose-1,8-P_2 + *altro*-heptulose-7-*P*	3.10
D-*glycero*-D-*altro*-Octulose-1,8-P_2 + *altro*-heptulose-7-*P*	0.90

Hexose 6-phosphate formation from octulose bisphosphate

In an attempt to ascertain the fates of the two isomers of octulose bisphosphate, which were found in the incubation mixture during the synthesis of hexose 6-phosphate from ribose 5-phosphate (Fig. 1 and Table 3), experiments were conducted which were aimed at detecting reactions resulting in hexose 6-phosphate formation from octulose bisphosphate. The results from these experiments are shown in Table 5.

A relatively high rate of hexose 6-phosphate production was observed with D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and erythrose 4-phosphate as substrates and this rate approached the maximum rate of hexose 6-phosphate production of 3.06nmol/min per mg of protein obtained with ribose 5-phosphate as the sole substrate for the rat liver enzyme preparation (Fig. 3). When the reaction was allowed to proceed to equilibrium with excess erythrose 4-phosphate, 2mol-equiv. of hexose 6-phosphate/mol-equiv. of octulose bisphosphate was formed. This stoicheiometry was consistent with the equation $C_8 + C_4 \rightarrow C_6 + C_6$ and was considered to be evidence in support of the sequential operation of a phosphatase or a phosphate-transferring enzyme (reaction 8) and transketolase (reaction 9):

D-*glycero*-D-*ido*-Octulose-1,8-P_2 → D-*glycero*-D-*ido*-octulose-8-P + P_i (8)

D-*glycero*-D-*ido*-Octulose-8-P + erythrose-4-P ⇄ fructose-6-P + glucose-6-P (9)

When both isomers of octulose bisphosphate were individually incubated with the rat liver enzyme preparation, hexose 6-phosphate was only produced at approx. 0.11–0.12-fold the rate obtained with erythrose 4-phosphate and D-*glycero*-D-*ido*-octulose 1,8-bisphosphate as substrates. If DL-glyceraldehyde 3-phosphate replaced erythrose 4-phosphate or D-*glycero*-D-*altro*-octulose 1,8-bisphosphate replaced D-*glycero*-D-*ido*-octulose 1,8-bisphosphate, the rate of production of hexose 6-phosphate was markedly decreased. The rate of hexose 6-phosphate production from D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and *altro*-heptulose 7-phosphate (Table 5) was 3.1nmol/min per mg of protein, and this result was concluded to be evidence for the operation of reactions (10), (11) and (9), where reaction (10) was catalysed by a phosphotransferase and reaction (11) by aldolase:

D-*glycero*-D-*ido*-Octulose-1,8-P_2 + *altro*-heptulose-7-P ⇌ D-*glycero*-D-*ido*-octulose-8-P + *altro*-heptulose-1,7-P_2 (10)

altro-Heptulose-1,7-P_2 ⇌ dihydroxyacetone-P + erythrose-4-P (11)

Transaldolase inhibition by arabinose 5-phosphate

The reaction mechanisms relocating ^{14}C from C-1 of ribose 5-phosphate to C-2 and C-6 of glucose 6-phosphate (Williams *et al.*, 1978) commenced to change at approx. 3h and were succeeded by reactions which then proceeded to locate ^{14}C in C-1 and C-3 of glucose 6-phosphate. As reactions that locate ^{14}C in C-1 and C-3 of glucose 6-phosphate can involve the sequential reactions catalysed by transketolase, transaldolase and transketolase (Horecker *et al.*, 1954), a regulatory metabolite of transaldolase was sought whose function could account for the observed change in ^{14}C distribution evident after 3h. Among the metabolites observed to accumulate and reach a maximum concentration during the first 30min to 3h of the 17h incubation were xylulose 5-phosphate, arabinose 5-phosphate, *altro*-heptulose 7-phosphate, *manno*-heptulose 7-phosphate, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and fructose 1,6-bisphosphate (Table 3). Arabinose 5-phosphate strongly inhibited transaldolase and thereby offered a means by which the distribution of ^{14}C into C-1 and C-3 may be suppressed during the first 3h of the incubation. We have reason to suspect from preliminary studies that *manno*-heptulose 7-phosphate may also be a regulatory inhibitor of liver transaldolase activity (P. F. Blackmore, unpublished result); however, we wish to refrain from making detailed comment until the data are unequivocally complete. In Fig. 4 the results from studies of the inhibitory kinetics of crystalline yeast transaldolase by arabinose 5-phosphate are shown. The double-reciprocal plot in Fig. 4(*a*) is for reaction (6), where the concentration of fructose 6-phosphate was saturating and the concentration of erythrose 4-phosphate was varied. From the intercept on the $1/s$ axis, K_m for erythrose 4-phosphate was found to be 23.5μM, which agreed with the value of 20μM reported by Pontremoli *et al.* (1960). Fig. 4(*a*) also shows the effect of three concentrations of arabinose 5-phosphate on the rates of the reaction. These results have been expressed in the form of a Dixon (1953) plot (Fig. 4*b*). From the common point of intersection in the negative quadrant, K_i was extrapolated and found to be 75μM and from the form of the Dixon plot it was concluded that arabinose 5-phosphate was a competitive inhibitor of the substrate erythrose 4-phosphate in reaction (6).

The double-reciprocal plot in Fig. 4(*c*) is for the reverse of reaction (6) in which *altro*-heptulose 7-phosphate was saturating and the concentration of glyceraldehyde 3-phosphate was varied. From the intercept on the $1/s$ axis, K_m for glyceraldehyde 3-phosphate was found to be 0.223mM which agreed with the value of 0.22mM reported by Horecker & Smyrniotis (1955). Fig. 4(*c*) also shows the effect of three concentrations of arabinose 5-phosphate on the reaction rates and these results have been expressed

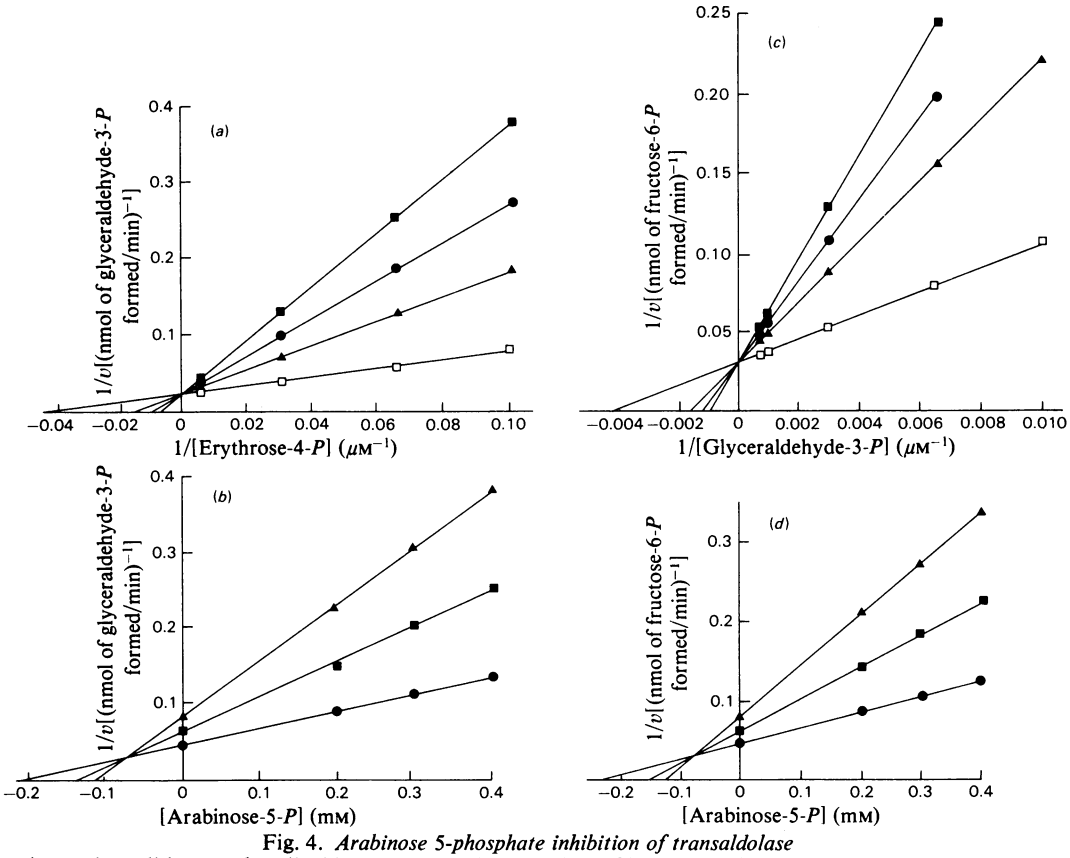


Fig. 4. *Arabinose 5-phosphate inhibition of transaldolase*

Experimental conditions as described in text. (*a*) Reciprocal plots of initial velocity, substrate concentration data are for reaction (6). The concentration of fructose 6-phosphate was saturating, and erythrose 4-phosphate concentration was varied. The effect of the following three concentrations of arabinose 5-phosphate, 0.4mM (■), 0.3mM (●), 0.2mM (▲) and none (□), on the initial velocity of reaction (6) are shown. (*b*) Dixon plot of the results of (*a*). (*c*) Reciprocal plots of initial velocity versus substrate concentration data are for reaction (6) studied in the reverse direction. The concentration of *altro*-heptulose 7-phosphate was saturating, and the concentration of glyceraldehyde 3-phosphate was varied. The effect of the following three concentrations of arabinose 5-phosphate, 0.4mM (■), 0.3mM (●), 0.2mM (▲) and none (□), on the initial velocity of reaction (6) studied in the reverse direction are shown. (*d*) Dixon plot of the results of (*c*).

in the form of a Dixon plot (Fig. 4*d*). From the common point of intersection the K_i was extrapolated and found to be approx. 70μM and from the form of the plot it was concluded that arabinose 5-phosphate was a competitive inhibitor of the substrate glyceraldehyde 3-phosphate in reaction (6).

Arabinose 5-phosphate and the transaldolase exchange reaction in vitro

Results in Fig. 4 show that arabinose 5-phosphate was a competitive inhibitor of glyceraldehyde 3-phosphate and erythrose 4-phosphate in reaction (6) which was catalysed by transaldolase. Transketolase and transaldolase both catalyse ^{14}C-exchange reactions *in vitro* (Clark *et al.*, 1971; Ljungdahl *et al.*, 1961). Since transaldolase is a component of the rat liver enzyme preparation (Williams *et al.*, 1978), the effect of an inhibitory concentration of arabinose 5-phosphate on the transaldolase-catalysed exchange reaction between fructose 6-phosphate and [^{14}C]glyceraldehyde 3-phosphate (reaction 12) was examined (Fig. 5):

$$[^{14}C]\text{Glyceraldehyde-3-}P + \text{fructose-6-}P \rightleftharpoons \text{glyceraldehyde-3-}P + [^{14}C]\text{fructose-6-}P \qquad (12)$$

Arabinose 5-phosphate at a concentration of 0.62mM (a concentration sufficient to produce approx. 50% inhibition of the mass-transferring reaction catalysed by transaldolase under the

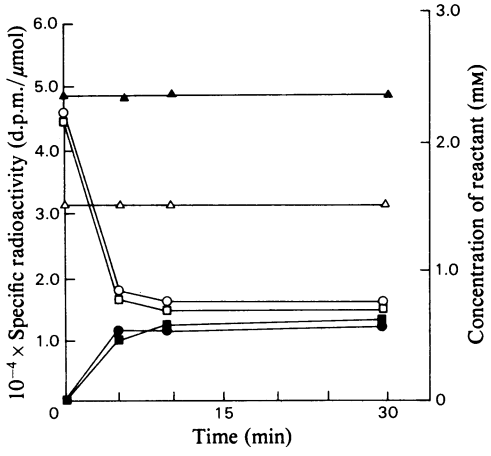


Fig. 5. *Transaldolase exchange reaction in the presence of arabinose 5-phosphate*

Each incubation contained in a volume of 4.2ml at 25°C: glycylglycine/KOH buffer, pH 7.4 (0.376mmol); fructose 6-phosphate (10μmol); [2-^{14}C]glyceraldehyde 3-phosphate (13μmol and 3 × 10^5 d.p.m.; see the Experimental section for preparation); 0.06 unit of transaldolase. Arabinose 5-phosphate (2.6μmol) was added only to the test mixture. Samples of 1.0ml were removed before the addition of the enzyme and at 5, 10 and 30min. Each sample together with 2 vol. of water washings was passed through a column (0.5cm × 10cm) of Dowex 50W (X8 resin; H^+ form). The eluate was concentrated to 0.1ml and applied as a discrete band to Whatman 3MM chromatography paper and the sugar phosphates were resolved in solvent system C. Each region of the chromatogram corresponding to fructose 6-phosphate and glyceraldehyde 3-phosphate was eluted from the paper and analysed for radioactivity and assayed for the particular sugar phosphate (Williams *et al.*, 1978). The results are means of duplicate determinations from two experiments and include the concentrations of glyceraldehyde 3-phosphate (△) and fructose 6-phosphate (▲) and the specific radioactivities of glyceraldehyde 3-phosphate (○, □) and fructose 6-phosphate (●, ■) in the presence and absence of arabinose 5-phosphate respectively.

conditions specified; J. F. Williams, P. F. Blackmore & M. G. Clark, unpublished result) was without effect on the transaldolase-catalysed exchange reaction (reactions 13, 14 and sum reaction 12).

Fructose-6-*P* + transaldolase ⇌ dihydroxyacetone–enzyme + glyceraldehyde-3-*P* (13)

[^{14}C]Glyceraldehyde-3-*P* + dihydroxyacetone–enzyme ⇌ [^{14}C]fructose-6-*P* + transaldolase (14)

The exchange reaction catalysed by transaldolase (reaction 12) proceeded at a rate 2-fold higher than the maximum rate for the forward transfer to acceptor (reaction 6) when measured by glyceraldehyde 3-phosphate formation.

Discussion

Since it was not possible to reconcile the data on the liver enzyme-catalysed distributions of ^{14}C in C-2 and C-6 of glucose 6-phosphate formed from [1-^{14}C]-ribose 5-phosphate by the accepted reaction scheme for the anaerobic segment of the pentose phosphate pathway (Williams *et al.*, 1978), the possibility existed that there were new reaction sequences for this metabolic pathway. The results reported here and in the previous paper (Williams *et al.*, 1978) may be interpreted in the framework of a new reaction scheme for the pentose phosphate pathway in liver (Schemes 1 and 2) and the discussion is directed to this end.

Initially reactions were sought which, individually or in combination with other known reactions, could account for the distribution of ^{14}C into C-2 and C-6 of glucose 6-phosphate formed at 1, 2, 5, 30min and 3h during the repeat of the Horecker *et al.* (1954) experiment (Williams *et al.*, 1978). The reactions examined included carboxylation, decarboxylation, formation of volatile or acid-volatile compounds and the incorporation of ^{14}C into glucose 6-phosphate from unlabelled ribose 5-phosphate and a variety of ^{14}C-labelled C_1, C_2 and C_3 compounds (Table 1). All the reactions examined were proved negative and provided no results that offered any lead in the further search for a new reaction pathway.

A chromatographic examination of the intermediates formed at various times throughout the 17h incubation by ion-exchange chromatography (Fig. 1) was conducted to search and attempt to account for the carbon deficit (as phosphorylated sugars) which was observed (Williams *et al.*, 1978). Five intermediates which had not previously been attributed to liver metabolism were isolated and identified. A re-evaluation of the carbon-balance data of Williams *et al.* (1978) to include data obtained for the concentration of the new additional intermediates revealed that more than 97% of the substrate (ribose 5-phosphate) carbon was accounted for at all times examined during the incubation period (Table 3). The additional intermediates were arabinose 5-phosphate, D-*glycero*-D-*altro*-octulose 1,8-bisphosphate, D-*glycero*-D-*ido*-octulose 1,8-bisphosphate, *manno*-heptulose 7-phosphate and *altro*-heptulose 1,7-bisphosphate. Dihydroxyacetone was also identified in samples removed from the reaction mixture after 3h, and was concluded to have arisen as a consequence of the high concentration of dihydroxyacetone phosphate present at this time (Table 3) and the relative ease with which the phosphate group is hydrolysed (Dawson *et al.*, 1969). The recalculated stoicheiometry for the conversion of 108μmol of ribose 5-phosphate into hexose 6-phosphate indicated that 95.8μmol of pentose 5-phosphate after a 17h reaction, formed 48.6μmol of hexose monophosphate,

26.9 μmol of triose phosphate (this value takes into account the dihydroxyacetone phosphate moiety of octulose, *altro*-heptulose and fructose bisphosphates, the glyceraldehyde 3-phosphate moiety of fructose 1,6-bisphosphate and the free dihydroxyacetone) and 14.1 μmol of heptulose 7-phosphate.

The presence of arabinose 5-phosphate in samples removed from the incubation of rat liver enzyme preparation with ribose 5-phosphate (Table 3) was, to our knowledge, the first report of the presence of this compound being formed by an enzyme or enzymes derived from a mammalian organism. Arabinose 5-phosphate was, however, identified by Scott & Cohen (1951) as one of the pentose 5-phosphates formed after the oxidative decarboxylation of 6-phosphogluconate by a crude yeast enzyme preparation.

[1-^{14}C]Arabinose is known to be metabolized by some mammalian tissues to give rise to $^{14}CO_2$, [^{14}C]lactate and [^{14}C]glucose (Segal & Foley, 1959; Stjernholm & Noble, 1963). From the studies of Stjernholm & Noble (1963), it was established that [1-^{14}C]arabinose was metabolized by a transketolase/transaldolase-catalysed sequence, in leucocytes, to form hexose; however, the exact mode of its conversion into a phosphorylated intermediate is not known. Stjernholm & Noble (1963) suggested two alternative pathways: (i) isomerization of arabinose to ribulose followed by phosphorylation to ribulose 5-phosphate and (ii) phosphorylation to arabinose 5-phosphate then isomerization to ribulose 5-phosphate. These two sequences would give rise to the same labelling patterns in hexoses, hence no distinction could be made at that time between these two alternatives. Segal & Foley (1959), from their studies in man, suggested that arabinose may be metabolized by the following sequence of reactions: arabinose $\rightarrow$ arabonic acid $\rightarrow$ 3-deoxy-2-oxoarabonic acid $\rightarrow$ pyruvic acid and glycollic acid, although no experimental evidence was provided for this pathway.

The results in Fig. 3 indicated that the rat liver enzyme preparation contained enzymes which interconverted arabinose 5-phosphate with ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate. The first compound to be detected by both chromatographic and enzymic analysis (Fig. 3) was ribose 5-phosphate. This observation was consistent with the presence of an enzyme, tentatively named arabinose phosphate 2-epimerase in the rat liver enzyme preparation. Although not named, a similar enzyme was reported by Levin & Racker (1959) for *Pseudomonas aeruginosa*, which also catalysed the conversion of arabinose 5-phosphate into ribose 5-phosphate. These results, however, do not rule out the possibility that there is another enzyme or enzymes present in rat liver for the interconversion of arabinose 5-phosphate to other pentose 5-phosphates. For example, there may be an enzyme present which reversibly interconverts arabinose 5-phosphate to ribulose 5-phosphate and is similar to the enzyme phosphoarabinoisomerase demonstrated to occur in the bacterium *Propionibacterium pentosaceum* by Volk (1959).

The data of Figs 2(*a*) and 2(*b*) and Table 4 provide evidence to indicate that arabinose 5-phosphate was utilized not only by the rat liver enzyme preparation but also by enzymes from several tissues of the rat to produce triose 3-phosphate and hexose 6-phosphate. These results contrast with those of Dickens (1938), who reported that arabinose 5-phosphate was not metabolized by laked horse blood, brain slices or yeast Lebedew fluid, since it did not affect the oxygen uptake by these tissues. Dickens (1938) concluded that arabinose 5-phosphate was not an intermediate of the pentose phosphate pathway.

The identification of D-*glycero*-D-*altro*-octulose 1,8-bisphosphate in samples of the rat liver enzyme incubation mixture provided evidence to suggest that this compound was formed in an aldolase-catalysed reaction involving ribose 5-phosphate and dihydroxyacetone phosphate as substrates (reaction 15).

$$\text{Ribose 5-phosphate} + \text{dihydroxyacetone-}P \rightleftharpoons \text{D-}glycero\text{-D-}altro\text{-octulose-1,8-}P_2 \quad (15)$$

The D-*glycero*-D-*ido*-octulose 1,8-bisphosphate found in samples of the rat liver enzyme incubation mixture may have resulted from either of two reactions: an octulose bisphosphate 5-epimerase-catalysed reaction from D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (reaction 16):

$$\text{D-}glycero\text{-D-}altro\text{-Octulose-1,8-}P_2 \rightleftharpoons \text{D-}glycero\text{-D-}ido\text{-octulose-1,8-}P_2 \quad (16)$$

or an aldolase-catalysed condensation of dihydroxyacetone phosphate with arabinose 5-phosphate (reaction 17):

$$\text{Arabinose-5-}P + \text{dihydroxyacetone-}P \rightleftharpoons \text{D-}glycero\text{-D-}ido\text{-octulose-1,8-}P_2 \quad (17)$$

The relatively high aldolase activity in the rat liver enzyme preparation favours the proposal that reaction (17) is a component of the new pentose phosphate pathway reaction scheme in liver.

The results of Table 5 for the rates of formation of glucose 6-phosphate and fructose 6-phosphate from each of the octulose bisphosphates and erythrose 4-phosphate were also inconsistent with the operation of an octulose bisphosphate 5-epimerase (reaction 16), for if such an enzyme was present, then both isomers would be able to readily interconvert and hence give rise to similar rates of hexose 6-phosphate production.

Since the rate of hexose 6-phosphate formation from the D-*glycero*-D-*ido*-octulose 1,8-bisphosphate isomer was 5.4-fold higher than that for the D-*glycero*-D-*altro* isomer, this is taken to be consistent with the operation of reaction (17) for the formation of

D-*glycero*-D-*ido*-octulose 1,8-bisphosphate in the rat liver enzyme preparation. Thus its formation was independent of the presence of the D-*glycero*-D-*altro* isomer but dependent on the formation of arabinose 5-phosphate from other pentose phosphates by the enzyme preparation.

Table 5 shows that (*a*) the rate of formation of glucose 6-phosphate and fructose 6-phosphate from D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and erythrose 4-phosphate (2.41 nmol/min per mg of protein) approached the maximum rate for the formation of hexose 6-phosphate from ribose 5-phosphate by the rat liver enzyme preparation (2.9 nmol/min per mg of protein; Williams *et al.*, 1978); (*b*) the enzymes responsible for hexose 6-phosphate production by the rat liver enzyme preparation in (*a*) were substrate-specific, since both isomers of octulose bisphosphate gave entirely different rates of hexose 6-phosphate production; (*c*) the stoicheiometry of the conversion in (*a*) when allowed to proceed to equilibrium was consistent with the equation: $C_8 + C_4 \rightarrow C_6 + C_6$ since 2 mol-equiv. of hexose 6-phosphate was produced and 1 mol-equiv. of D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and 1 mol-equiv. of erythrose 4-phosphate was utilized (J. F. Williams, P. F. Blackmore & M. G. Clark, unpublished results). The observations (*a*) and (*b*) suggest that octulose bisphosphate and in particular D-*glycero*-D-*ido*-octulose 1,8-bisphosphate may be an intermediate in the formation of hexose 6-phosphate from ribose 5-phosphate by the rat liver enzyme preparation. The possible formation of glucose 6-phosphate and fructose 6-phosphate from the aldolase-catalysed cleavage products of D-*glycero*-D-*ido*-octulose 1,8-bisphosphate (reactions 18, 19 and 20) was discounted by the measured stoicheiometry (*c*) and observed specificity (*b*) of the reaction (Table 5).

D-*glycero*-D-*ido*-Octulose-1,8-*P*$_2$ $\rightleftharpoons$ dihydroxyacetone-*P* + arabinose-5-*P* (18)

Arabinose-5-*P* ------$\rightarrow$ xylulose-5-*P* (19)

Xylulose-5-*P* + erythrose-4-*P* $\rightleftarrows$ fructose-6-*P* + glyceraldehyde-3-*P* (20)

The stoicheiometry of reactions (18), (19) and (20) is $C_8 + C_4 \rightarrow C_6 + 2C_3$, and, since hexose phosphatase is absent from the rat liver enzyme preparation (Horecker *et al.*, 1954; Williams *et al.*, 1978), only one half of the expected yield of hexose 6-phosphate would be formed from reactions (18), (19) and (20).

Phosphotransferase-catalysed reactions

The results from Table 5 imply that D-*glycero*-D-*ido*-octulose 1,8-bisphosphate gave rise initially to D-*glycero*-D-*ido*-octulose 8-phosphate in a reaction catalysed by a phosphatase reaction (reaction 8) in the rat liver enzyme preparation. The resultant D-*glycero*-D-*ido*-octulose 8-phosphate, which is a substrate for transketolase, would then be available to react in a transketolase-catalysed reaction with erythrose 4-phosphate to form glucose 6-phosphate and fructose 6-phosphate (reaction 9). As *altro*-heptulose 1,7-bisphosphate was detected in early-time-interval samples taken from the incubation mixture composed of rat liver enzyme preparation and ribose 5-phosphate, and since neither erythrose 4-phosphate nor products of the transaldolase-catalysed reaction (i.e. [1,3-^{14}C]hexose 6-phosphate) were found in these samples, it appeared feasible that *altro*-heptulose 1,7-bisphosphate had been formed from *altro*-heptulose 7-phosphate by phosphotransferase catalysis (reaction 10).

The dual role of an enzyme being both a phosphatase and a phosphotransferase is consistent with the observations of Morton (1958*a*,*b*,*c*). The comments made by Morton (1958*a*) regarding the kinetics of hydrolysis of phenyl phosphate by alkaline phosphatase may well apply to the phosphotransferase proposed to exist by us in the rat liver enzyme preparation: 'It therefore appeared possible that conditions could be chosen such that any new ester synthesized by a phosphotransferase reaction (21) would be only slowly hydrolysed by the phosphatase (reaction 22), whereas hydrolysis of the initial substrate by the phosphatase (reaction 23) would proceed quite rapidly'.

$$RO\cdot PO_3^{2-} + R'O\cdot H \rightarrow RO\cdot H + R'O\cdot PO_3^{2-} \quad (21)$$

$$R'O\cdot PO_3^{2-} + HO\cdot H \rightarrow R'O\cdot H + HO\cdot PO_3^{2-} \quad (22)$$

$$RO\cdot PO_3^{2-} + HO\cdot H \rightarrow RO\cdot H + HO\cdot PO_3^{2-} \quad (23)$$

For the phosphotransferase under consideration in this paper R is D-*glycero*-D-*ido*-octulose 8-phosphate and R′ is *altro*-heptulose 7-phosphate, and transfer of the C-1 phosphate group of D-*glycero*-D-*ido*-octulose 1,8-bisphosphate ($RO\cdot PO_3^{2-}$) is achieved by reaction (21).

The date of Table 5 indicate that hexose 6-phosphate was formed from D-*glycero*-D-*ido*-octulose 1,8-bisphosphate and *altro*-heptulose 7-phosphate by the rat liver enzyme preparation at a rate comparable with the maximum rate of hexose 6-phosphate formation.

Since D-*glycero*-D-*altro*-octulose 1,8-bisphosphate cannot replace D-*glycero*-D-*ido*-octulose 1,8-bisphosphate in reaction (11), it was concluded that the production of hexose 6-phosphate was the result of a phosphotransferase-catalysed reaction, of the form of reaction (21) and not the result of an aldolase-, triose phosphate isomerase- and transaldolase-catalysed sequence of reactions (18), (24) and (25).

Dihydroxyacetone-*P* $\rightleftharpoons$ glyceraldehyde-3-*P* (24)

altro-Heptulose-7-*P* + glyceraldehyde-3-*P* $\rightleftharpoons$ fructose-6-*P* + erythrose-4-*P* (25)

The failure to detect D-*glycero*-D-*ido*-octulose 8-phosphate in samples of the incubation (Table 3) is consistent with the phosphotransferase-catalysed reaction being a rate-limiting reaction in the overall pathway in the formation of hexose 6-phosphate from pentose 5-phosphate.

A small but significant rate of hexose 6-phosphate production was observed with *altro*-heptulose-7-P when incubated alone with the rat liver enzyme preparation (Table 5). As the commercial *altro*-heptulose 7-phosphate had been purified to remove contaminating pentose 5-phosphate and hexose 6-phosphates (see the Experimental section), this small rate was evidence that reaction (26) (sum of partial reactions 26a, 26b, 26c, and 26d), which is catalysed by transketolase and transaldolase, was operative in the rat liver enzyme preparation. From the known substrate specificity of transketolase and transaldolase (Racker, 1961*a*,*b*) and the fact that transketolase and transaldolase acting together are known to catalyse a similar reaction but with fructose 6-phosphate as substrate (Bonsignore *et al.*, 1962), reaction 26 should theoretically be catalysed by these enzymes in the rat liver enzyme preparation.

altro-Heptulose-7-*P* + transketolase ⇌ glycolaldehyde − transketolase + ribose 5-P (26a)

altro-Heptulose-7-*P* + transaldolase ⇌ dihydroxyacetone − transaldolase + erythrose-4-*P* (26b)

Erythrose-4-*P* + glycolaldehyde − transketolase ⇌ fructose-6-*P* + transketolase (26c)

Ribose-5-*P* + dihydroxyacetone − transaldolase ⇌ D-*glycero*-D-*altro*-octulose-8-*P* + transaldolase (26d)

2 *altro*-Heptulose-7-*P* ⇌ fructose-6-*P* + D-*glycero*-D-*altro*-octulose-8-*P* (26)

Enzyme-catalysed ^{14}C-exchange reactions of the pentose phosphate pathway

From studies on the metabolism of [2-^{14}C]glucose and [1-^{14}C]ribose by rabbit liver *in situ*, Williams *et al.* (1971) concluded that the main reactions contributing to the relocation of ^{14}C were the exchange reactions catalysed by the two group-transferring enzymes of the pentose phosphate pathway, transketolase and transaldolase. The reactions were proposed to be both extensive (owing to the board substrate specificity of transketolase and transaldolase) and rapid.

The results of Clark *et al.* (1971) and Ljungdahl *et al.* (1961) showed that both transketolase- and transaldolase-catalysed ^{14}C-exchange reactions *in vitro*. From these studies it was apparent that for each of the enzymes transketolase and transaldolase, a relatively stable enzyme–glycoaldehyde or enzyme–dihydroxyacetone complex (respectively) exists. The dissociating phosphorylated aldehyde is free to exchange with either like (e.g. erythrose 4-phosphate and [^{14}C]erythrose 4-phosphate) or unlike (e.g. erythrose 4-phosphate and glyceraldehyde 3-phosphate either labelled or unlabelled) aldehydes. ^{14}C label may then be introduced into or removed from the top enzyme-bound fragment of the sugar molecule or the lower phosphorylated aldehyde.

Since distribution patterns of ^{14}C in glucose 6-phosphate consistent with the ordered reaction sequence catalysed by transketolase, transaldolase and transketolase only became evident after 3h (Williams *et al.*, 1978), it is necessary to explain the mechanisms that allow the labelling of C-2 and C-6 of hexose 6-phosphate during the early time course (up to 3h) and then permit the change to C-1 and C-3 labelling during the remaining 14h. It is proposed that the relatively high concentration of arabinose 5-phosphate present in the early time intervals of the incubation (1.77mM at 30min and 0.57mM at 3h; Table 3) was sufficient to inhibit the transaldolase-catalysed reaction between *altro*-[1,3-^{14}C]-heptulose 7-phosphate and glyceraldehyde 3-phosphate (reaction 27), the *altro*-[1,3-^{14}C]heptulose 7-phosphate being formed from [1-^{14}C]ribose 5-phosphate and [1-^{14}C]xylulose 5-phosphate in a transketolase reaction (reaction 28).

altro-[1,3-^{14}C]Heptulose-7-*P* + glyceraldehyde-3-*P* ⇌ [1,3-^{14}C]fructose-6-*P* + erythrose-4-*P* (27)

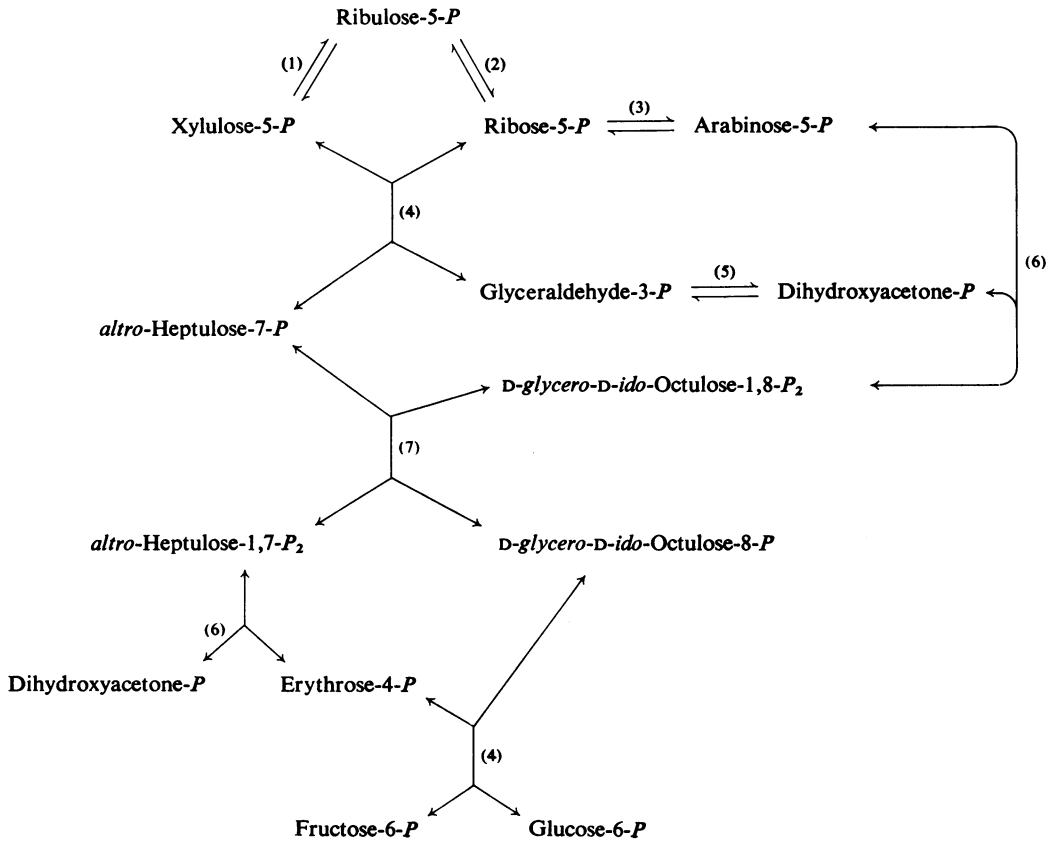
[1-^{14}C]Ribose-5-*P* + [1-^{14}C]xylulose-5-*P* ⇌ *altro*-[1,3-^{14}C]heptulose-7-*P* + glyceraldehyde-3-*P* (28)

Reaction (27) which forms [1,3-^{14}C]fructose 6-phosphate is inhibited (K_i for arabinose 5-phosphate was 70μM; Fig. 5) until such time that the concentration of arabinose 5-phosphate is decreased, i.e. time intervals after 3h. In contrast, the capacity for exchange catalysis by transaldolase (Fig. 5 and reaction 12) remained quite active, even in the presence of concentrations of arabinose 5-phosphate sufficiently high to significantly inhibit the mass-transfer reaction catalysed by this enzyme.

The ^{14}C content of C-6 only significantly decreased after 8h (Table 5, Williams *et al.*, 1978), which supports the conclusion that concomitant with the operation of the transaldolase-catalysed mass-transfer reaction there is a decrease in the contribution of the exchange reaction (12) and hence its contribution to the labelling pattern of hexose 6-phosphate.

A new non-oxidative pentose phosphate pathway

The reaction sequences shown in Scheme 1 and Scheme 2 will theoretically account for the ^{14}C label distributions found in the glucose 6-phosphate formed during the initial phase (1min to 3h) of the repeat Horecker *et al.* (1954) experiment (Williams *et al.*, 1978). Scheme 1 comprises carbon-flux reactions which are proposed to account for the synthesis of



Scheme 1. *Proposed reaction sequence for the new non-oxidative pentose phosphate pathway*
The reactions are catalysed by: ribulose phosphate 3-epimerase (5.1.3.1) (1); ribose phosphate isomerase (5.3.1.6) (2); arabinose phosphate 2-epimerase (3); transketolase (2.2.1.1) (4); triose phosphate isomerase (5.3.1.1) (5); aldolase (4.1.2.13) (6); a phosphotransferase (7).

glucose 6-phosphate and fructose 6-phosphate from ribose 5-phosphate catalysed by the rat liver enzyme preparation. The formulation of this scheme arose from the considerations discussed above.

Scheme 2 details the ^{14}C-transferring reactions of the new pentose phosphate pathway and is proposed to account for the formation of [2,6-^{14}C]-hexose 6-phosphate from [1-^{14}C]ribose 5-phosphate. The reactions in Scheme 2 incorporate all of the reactions of Scheme 1 and some of the transketolase- and transaldolase-catalysed exchange reactions demonstrated to occur *in vitro*, see, e.g. Clark *et al.* (1971).

The mechanism for the incorporation of ^{14}C into C-6 of glucose 6-phosphate (Williams *et al.*, 1978) may be explained by a transaldolase-catalysed exchange reaction (Scheme 2, reaction P). The path of ^{14}C catalysed by these exchange reactions involves the following. In Scheme 2, reactions D and L form two differently ^{14}C-labelled species of *altro*-heptulose 7-phosphate, the *altro*-[1,3-^{14}C]heptulose 7-phosphate (reaction D) being formed in a transketolase-catalysed reaction [a reaction in the Horecker *et al.* (1954) reaction sequence] and the *altro*-[1-^{14}C]heptulose 7-phosphate being formed by a combination of the transketolase exchange and transaldolase exchange reactions (reactions K and L respectively). After phosphotransferase catalysis of the two ^{14}C-labelled species of *altro*-heptulose 7-phosphate (namely [1,3-^{14}C]- and [1-^{14}C]-) two correspondingly labelled species of *altro*-heptulose 1,7-bisphosphate are formed. When *altro*-heptulose 1,7-bisphosphate is cleaved in an aldolase-catalysed reaction (reaction H) to form erythrose 4-phosphate and dihydroxyacetone phosphate, label is introduced into dihydroxyacetone phosphate ([3-^{14}C]- or [1,3-^{14}C]-dihydroxyacetone phosphate) and then into [3-^{14}C]- and [1,3-^{14}C]-glyceraldehyde 3-phosphate respectively by a triose phosphate isomerase-catalysed reaction (reaction E). In reaction M or sum reaction P the formation of [6-^{14}C]fructose 6-phosphate from [3-^{14}C]glyceralde-

Scheme 2. *^{14}C-transferring reactions of the proposed new pentose phosphate pathway*

Abbreviations: Rbu, ribulose; Xlu, xylulose; Ara, arabinose; Rib, ribose; Ery, erythrose; Fru, fructose; Glc, glucose; Gra, glyceraldehyde; Grn, dihydroxyacetone; Oct, D-*glycero*-D-*ido*-octulose; Seh, sedoheptulose (*altro*-heptulose); TKE, transketolase exchange reaction; TAE, transaldolase exchange reaction.

[1-^{14}C]Rib-5-*P*	$\rightleftharpoons$	[1-^{14}C]Rbu-5-*P*	A
[1-^{14}C]Ru-5-*P*	$\rightleftharpoons$	[1-^{14}C]Xlu-5-*P*	B
[1-^{14}C]Rib-5-*P*	$\rightleftharpoons$	[1-^{14}C]Ara-5-*P*	C
[1-^{14}C]Xlu-5-*P* + [1-^{14}C]Rib-5-*P*	$\rightleftharpoons$	[1,3-^{14}C]Seh-7-*P* + Gra-3-*P*	D
Gra-3-*P*	$\rightleftharpoons$	Grn-*P*	E
[1-^{14}C]Ara-5-*P* + Grn-*P*	$\rightleftharpoons$	[4-^{14}C]Oct-1,8-P_2	F
[1,3-^{14}C]Seh-7-*P* + [4-^{14}C]Oct-1,8-P_2	$\longrightarrow$	[1,3-^{14}C]Seh-1,7-P_2 + [4-^{14}C]Oct-8-*P*	G
from L [1-^{14}C]Seh-7-*P*	$\longleftarrow$	[1-^{14}C]Seh-1,7-P_2	
[1,3-^{14}C]Seh-1,7-P_2	$\longrightarrow$	Ery-4-*P* + [1,3-^{14}C]Grn-*P*	H
[1-^{14}C]Seh-1,7-P_2	$\longleftarrow$	[3-^{14}C]Grn-*P*	
[4-^{14}C]Oct-8-*P* + Ery-4-*P*	$\rightleftharpoons$	[2-^{14}C]Glc-6-*P* + Fru-6-*P*	I
[2-^{14}C]Glc-6-*P*	$\rightleftharpoons$	[2-^{14}C]Fru-6-*P*	J
[1,3-^{14}C]Seh-7-*P* + [2-^{14}C]Fru-6-*P*	$\rightleftharpoons$	[2,3-^{14}C]Seh-7-*P* + [1-^{14}C]Fru-6-*P*	K
[2,3-^{14}C]Seh-7-*P* + [1-^{14}C]Fru-6-*P*	$\rightleftharpoons$	[2,3-^{14}C]Fru-6-*P* + [1-^{14}C]Seh-7-*P*	L
[3-^{14}C]Gra-3-*P* + [2,3-^{14}C]Fru-6-*P*	$\rightleftharpoons$	[2,3,6-^{14}C]Fru-6-*P* + Gra-3-*P*	M
[1-^{14}C]Seh-7-*P* + [2,3,6-^{14}C]Fru-6-*P*	$\rightleftharpoons$	[1,6-^{14}C]Fru-6-*P* + [2,3-^{14}C]Seh-7-*P*	N
[2,3-^{14}C]Seh-7-*P* + [1,6-^{14}C]Fru-6-*P*	$\rightleftharpoons$	[2,6-^{14}C]Fru-6-*P* + [1,3-^{14}C]Seh-7-*P*	O
Sum of reactions K, L, M, N and O			
[2-^{14}C]Fru-6-*P* + [3-^{14}C]Gra-3-*P*	$\rightleftharpoons$	[2,6-^{14}C]Fru-6-*P* + Gra-3-*P*	P

hyde 3-phosphate is shown. If [1,3-^{14}C]glyceraldehyde 3-phosphate had been a reactant in the transaldolase exchange reaction (reaction P), then [4,6-^{14}C]fructose 6-phosphate and [4,6-^{14}C]glucose 6-phosphate (reaction J) would be formed. Only in glucose 6-phosphate sampled during the first min of the 'repeat' experiment (Williams *et al.*, 1978) was C-4 significantly labelled (10.1%) and hence the extent of the reactions leading to [1,3-^{14}C]glyceraldehyde 3-phosphate after 1 min must be small.

Since the exchange reactions in Scheme 2 are reversible, the products of the reactions K and L, namely [2,3-^{14}C]fructose 6-phosphate and *altro*-[1-^{14}C]heptulose 7-phosphate, may undergo the reverse reactions (reactions N and O). The transaldolase exchange reaction between like dihydroxyacetone acceptors (reaction M) may proceed at any stage throughout the series of exchanges without ever affecting the labelling patterns of C-1, C-2 and C-3 of the reactants, since the ^{14}C located in C-6 will not be changed by transketolase- and/or transaldolase-catalysed reaction (reactions K, L, N and O) using 'unlike' acceptors or donors. Because C-1 and C-3 of glucose 6-phosphate were not significantly labelled until 3 h, the rate of turnover of any [1-^{14}C]- and [3-^{14}C]-fructose 6-phosphate formed by reactions K and L and used in the reverse of reactions N and O must be considered to have been very rapid. The label in C-2 of fructose 6-phosphate will not be affected by the transketolase- and transaldolase-catalysed exchange reactions. The like dihydroxyacetone or glycolaldehyde acceptors mentioned above refer to the situation when the same aldehyde species exchange, one being labelled and the other unlabelled, e.g. glyceraldehyde 3-phosphate and [^{14}C]glyceraldehyde 3-phosphate, whereas unlike dihydroxyacetone or glycolaldehyde acceptors refer to the case when the two aldehydes are different and either one or both are ^{14}C-labelled, e.g. ribose 5-phosphate and [^{14}C]erythrose 4-phosphate.

The conclusions reached by Horecker *et al.* (1954) and Gibbs & Horecker (1954) from results of their experiments involving the [1-^{14}C]ribose 5-phosphate and [2,3-^{14}C]ribose 5-phosphate respectively by enzymes prepared from liver (Horecker *et al.*, 1954) and pea root and pea leaf tissues (Gibbs & Horecker, 1954) are now discussed with the view to suggesting that these results (Table 6) may be more easily interpreted by the reaction scheme of the new pentose phosphate pathway (Fig. 1).

In the experiments of Horecker *et al.* (1954) and Gibbs & Horecker (1954) the initial attempt to correlate experimental data with theoretical patterns of ^{14}C incorporation into glucose 6-phosphate by a transketolase (reaction 29), transaldolase (reaction 30)-catalysed sequence:

[1-^{14}C]Xylulose-5-*P* + [1-^{14}C]ribose-5-*P* $\rightleftharpoons$ *altro*-[1,3-^{14}C]heptulose-7-*P* + glyceraldehyde-3-*P* (29)

altro-[1,3-^{14}C]Heptulose-7-*P* + glyceraldehyde-3-*P* $\rightleftharpoons$ [1,3-^{14}C]fructose-6-*P* + erythrose-4-*P* (30)

was unsuccessful. Such a sequence led to a theoretical pattern where all of the ^{14}C was equally distributed between C-1 and C-3 of hexose 6-phosphate. A number of alternative reaction pathways were then pro-

Table 6. *Degradation data of Horecker et al.* (1954) *and Gibbs & Horecker* (1954), *contrasted with theoretical distribution patterns in glucose 6-phosphate for a variety of reaction pathways*

The transketolase (TK), transaldolase (TA) reaction sequence refers to reactions (29) and (30), and the TK, TA, TK reaction sequence refers to the sequential operation of reactions (29), (30) and (31). The fructose 1,6-bisphosphatase (FDPase) reaction applies to the situation when 2mol of triose phosphate, formed in reaction (31), react with aldolase to form fructose 1,6-bisphosphate which is then hydrolytically cleaved to form fructose 6-phosphate by the action of FDPase. The phosphotransferase reaction here refers to the case in which the phosphate from C-1 of fructose 1,6-bisphosphate is transferred to 'a suitable acceptor'.

	Substrate [1-^{14}C]ribose 5-phosphate							
	Experimentally found in:							
Carbon number of glucose-6-*P*	Pea leaf (2h)		Pea root (4h)	Rat liver (17h)	TK, TA	TK, TA, TK	TK, TA, TK FDPase	TK, TA, TK phosphotransferase
1	63	63	70	74	50	66.6	66.6	66.6
2	0	0	0	0	0	0	0	0
3	17	19	29	24	50	33.3	33.3	33.3
4	9	9	1	1	0	0	0	0
5	0	0	0	0	0	0	0	0
6	8	9	0	1	0	0	0	0
	Substrate [2,3-^{14}C]ribose 5-phosphate							
	Pea leaf (1h)		Pea root (4h)*	Rat liver (17h)				
1	3		4	7	0	0	0	0
2	13		25	28	50	40	33.3	33.3
3	36		27	20	0	20	25	25
4	43		42	45	50	40	41.7	41.7
5	5		2	1	0	0	0	0
6	0		0	0	0	0	0	0

* Temperature of incubation was 37°C, whereas all other incubations were at 25°C.

posed to explain the experimental labelling patterns (Table 6). The inclusion of a further transketolase reaction (reaction 31) utilizing erythrose 4-phosphate (the product of reaction 30) and [1-^{14}C]xylulose 5-phosphate offered the best and simplest explanation of the results obtained for the distribution of ^{14}C in glucose 6-phosphate formed from [1-^{14}C]ribose 5-phosphate in liver and pea root tissue (Table 6).

[1-^{14}C]Xylulose-5-*P* + erythrose-4-*P* $\rightleftharpoons$ [1-^{14}C]-fructose-6-*P* + glyceraldehyde-3-*P* (31)

Since the proposed transketolase, transaldolase, transketolase sequence is now conventionally accepted as the group-transferring mechanism for the pentose phosphate pathway, it is necessary to bring attention to the discrepancy between the experimental results obtained with [1-^{14}C]ribose 5-phosphate and those theoretically predicted (Table 6). The discrepancy concerns the ratio of the radioactivity in C-1/C-3 which was experimentally found to be 3.1 instead of the theoretical 2.0 for liver, 2.3 for pea root and 3.9 and 3.22 for pea leaf. There was also significant labelling of C-4 and C-6 when the reactions were catalysed by enzymes prepared from pea leaf. The difference between experiment and theory in the C-1/C-3 radioactivity ratios is of concern because the theoretical work which forms the foundation of all of the methods used to quantitatively measure the contributions of the pentose phosphate cycle to glucose metabolism (Wood & Katz, 1958), depends on the above C-1/C-3 ratio being exactly 2.0. The finding that this ratio was not achieved in the original experiments weakens the theoretical methods that depend on this feature (Katz & Wood, 1960; Wood *et al.*, 1963; Landau *et al.*, 1964; Landau & Katz, 1964, 1965; Katz & Wals, 1972).

In experiments where [2,3-^{14}C]ribose 5-phosphate was the substrate, there was no satisfactory agreement between the theoretical distributions of ^{14}C by the sequence of reactions (28), (30) and (31) and the experimental results (Table 6). Theoretically the group-transferring reactions catalysed by the transketolase, transaldolase and transketolase distribute isotope into glucose 6-phosphate with C-2 and C-4 equally labelled 40% and C-3 labelled 20%. Experimentally the ^{14}C radioactivity in C-2 varied from 28 to 13% for liver, pea root and pea leaf and that in C-3 ranged over 20, 27 and 36% for the same tissues; the C-4 values fitted the predicted values and ranged from 42 to 45%. No satisfactory explanation for the radioactivity consistently found in C-1 or C-5 of the glucose 6-phosphate formed in these experiments

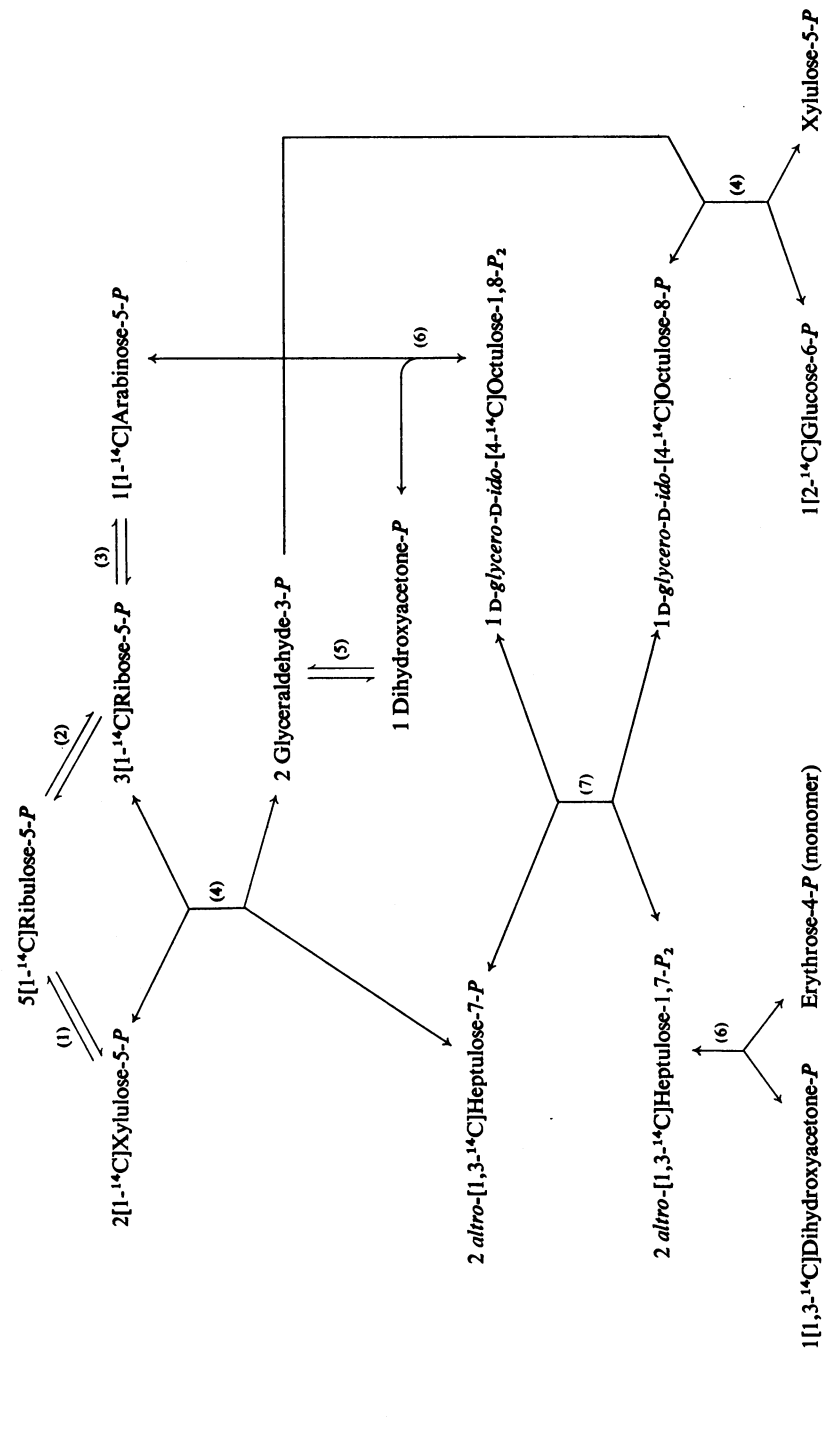
is possible by the presently accepted reaction sequence of the pentose pathway. Metabolism of [2,3-^{14}C]ribose 5-phosphate in the 'new pentose phosphate pathway' scheme (Scheme 1) theoretically causes C-1 of glucose 6-phosphate to be labelled. The data of the [2,3-^{14}C]ribose 5-phosphate experiments were difficult for the original workers to interpret and necessitated different explanations, depending on the tissue being considered. In all cases it was necessary that [1-^{14}C]triose 3-phosphate was formed and converted into [3,4-^{14}C]fructose 1,6-bisphosphate which was then acted on by fructose 1,6-bisphosphatase or a phosphotransferase to form [3,4-^{14}C]-fructose 6-phosphate. Fructose bisphosphatase was initially inhibited in liver enzyme preparation (Horecker *et al.*, 1954) (Williams *et al.*, 1978) and hence it was necessary for Gibbs & Horecker (1954) to propose a hypothetical phosphotransferase. A phosphotransferase-catalysed reaction is also proposed in the reaction sequence of the new pentose phosphate pathway. However, the fructose 1,6-bisphosphatase was quite active in the pea root and pea leaf tissues, and, although its inclusion contradicted the proposed mechanism, its operation was accepted in these tissues as a necessary reaction to allow an explanation of part of the labelling patterns. In the labelling patterns obtained with pea leaf (Table 6), Gibbs & Horecker (1954) found it necessary to propose that the ^{14}C-labelled triose phosphate was formed by aldolase cleavage of *altro*-heptulose 1,7-bisphosphate. In the present paper we report this reaction in the new pentose phosphate pathway scheme and in photosynthetic tissue (Clark *et al.*, 1974).

It is our conclusion that the reaction sequences of the new pentose phosphate pathway (Schemes 1 and 2) will: (1) account for the various distributions of ^{14}C in glucose 6-phosphate formed from [1-^{14}C]ribose 5-phosphate by liver enzymes (Williams *et al.*, 1978) during the time course of the repeat and extension of the original experiment which was used to establish the mechanism of the pentose pathway; (2) overcome some of the difficulties which arise in an explanation of some of the results of Horecker *et al.* (1954) and Gibbs & Horecker (1954); (3) afford a more reasoned and satisfactory explanation of the anomalous ^{14}C-labelling patterns found by us (Williams *et al.*, 1971) in hexose 6-phosphates after metabolism of [2-^{14}C]glucose and [1-^{14}C]ribose in liver *in situ*; (4) account for the failure to measure, by the methods of Katz & Wood (1960), a quantitatively significant contribution of pentose phosphate pathway to liver metabolism (Williams *et al.*, 1971; Hostetler & Landau, 1967) in spite of the presence of high activities of the enzymes conventionally assigned to the pathway (Novello & McLean, 1968). It has also been found that inclusion of the reactions of the anaerobic segment of the new pentose phosphate pathway in a scheme for the path of carbon in photosynthesis (Clark *et al.*, 1974) afforded an explanation of the 'Gibbs Effects' (Stiller, 1962) in photosynthesis.

Rat epididymal fat-pad is the only tissue that we have examined (Williams *et al.*, 1974) where metabolism of [2-^{14}C]glucose produced ^{14}C labelling of glucose 6-phosphate that matched in an unqualified way the predictions of a pentose phosphate pathway based on the tentative suggestion of Horecker *et al.* (1954).

It is suggested that one of the dominant factors which acts to strongly influence the fates of the individual carbon atoms that serve as diagnostic markers of the pentose phosphate pathway mechanism is aldolase activity. This suggestion is supported by our finding that the location of ^{14}C in glucose 6-phosphate formed in adipose-tissue metabolizing [2-^{14}C]glucose agreed almost exactly with the prediction of the pentose phosphate pathway (Horecker *et al.*, 1954). The activity of aldolase in adipose tissue (37nmol/min per mg of protein; Shafrir *et al.*, 1970) is approximately the same as the activity of transketolase and transaldolase (22 and 30nmol/min per mg of protein respectively; Novello & McLean, 1968) and the activity of aldolase (either ketose 1-phosphate aldolase or fructose bisphosphate aldolase) in liver *in vivo* and *in vitro* is 2.5 times greater than that found in adipose tissue (Knox, 1972; Williams *et al.*, 1974). The ratio of aldolase to either transketolase or transaldolase in liver is 6.75:1 (Novello & McLean, 1968). Very high ratios of aldolase to transaldolase varying from 50–300 to 1 for a variety of photosynthetic tissues are also reported (Peterkofsky & Racker, 1961). Davies (1961) has previously proposed that aldolase makes a contribution to the broad-specificity catalysis of group-transferring reactions in the anaerobic reaction sequence of the pentose phosphate pathway.

Finally we suggest that the different octulose bisphosphates formed in the new pentose phosphate pathway (Scheme 1) by liver *in vitro* and *in vivo* (P. F. Blackmore & J. F. Williams, unpublished results) may serve as potential reserves of ribose 5-phosphate (from D-*glycero*-D-*altro*-octulose 1,8-bisphosphate) and arabinose 5-phosphate (from D-*glycero*-D-*ido*-octulose 1,8-bisphosphate) because of the reactivities of these bisphosphates with aldolase. Our failure to be able to detect erythrose 4-phosphate in the experiments reported here (Williams *et al.*, 1978) supports the suggestion (Horecker & Mehler, 1955) that D-*altro*-heptulose 1,7-bisphosphate may serve as a cellular reserve for erythrose 4-phosphate. The method used to measure erythrose 4-phosphate in tissues (Srere *et al.*, 1958; Racker, 1965; Gumaa & McLean, 1969) has involved the transaldolase-catalysed transfer of a C_3 moiety from fructose 6-phosphate to erythrose 4-phosphate,



Scheme 3. *Reaction sequences of the new non-oxidative pentose phosphate pathway showing the influence of an alternative transketolase-catalysed reaction which will account for the observed decline of the specific radioactivity of pentose 5-phosphate (see Fig. 2 of Williams et al., 1978) during the time course of glucose 6-phosphate formation from* [1-^{14}C]*ribose 5-phosphate by rat liver enzyme preparation*

The enzymes are as described in the legend to Scheme 1.

generating sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate (Venkataraman & Racker, 1961). The assay is not specific for erythrose 4-phosphate, since *aldo* sugar phosphates with 3, 4 and 5 carbon atoms will serve as acceptors for the ketol unit (Venkataraman & Racker, 1961). For these reasons we feel that the concentration of erythrose 4-phosphate found in tissues [ascites cells, 0.036 μmol/g of cells (Gumaa & McLean, 1969); liver, 4.0nmol/g wet wt. (Greenbaum *et al.*, 1971); synaptosomes from brain (71 μmol/kg of protein) and brain homogenate (41 μmol/kg of protein) (Kauffman & Hakonen, 1977)] by using the above method may exaggerate the true concentrations of this intermediate. The method used to search for and measure erythrose 4-phosphate in this study (see preceding paper, Williams *et al.*, 1978) is specific and unambiguous. The very low amounts of erythrose 4-phosphate encountered in the experiments reported here (too low to be measured) are consistent with the earlier observations of Dische *et al.* (1960) and Dische & Igals (1961) on ribose 5-phosphate metabolism by blood haemolysates. Erythrose 4-phosphate was not identified by Horecker & Smyrniotis (1953) during their initial observations with transaldolase. It was the availability of synthetic erythrose 4-phosphate (Ballou *et al.*, 1955) which allowed Kornberg & Racker (1955) to establish the status of reaction 30 and to identify its reactants. In liver (Gumaa & McLean, 1968) and in erythrocytes (Bartlett & Bucolo, 1968) the concentration of erythrose 4-phosphate was too low to be detected, although all other intermediates and products of pentose 5-phosphate metabolism were found. The failure to consistently detect this intermediate in tissues may be due not only to its considerable reactivity and turnover after formation by reactions catalysed by transaldolase, transketolase and aldolase but to its existence as dimers (Blackmore *et al.*, 1976). Proton, ^{13}C and ^{31}P n.m.r. spectra of synthetic erythrose 4-phosphate (Ballou *et al.*, 1955; Simpson *et al.*, 1966) at pH 1 and 6 (25°C) showed the presence of three erythrose 4-phosphate dimers and erythrose 4-phosphate *gem*-diol. The *gem*-diol contributed 42% to the total concentration of the forms of erythrose 4-phosphate found; but the free aldehyde has not been detected (J. F. Williams, J. K. MacLeod & C. Duke, unpublished results).

The affinity of erythrose 4-phosphate for transketolase and transaldolase (Dische & Igals, 1961; Pontremoli *et al.*, 1960; Datta & Racker, 1961) may also act to sequester erythrose 4-phosphate to the enzymes involved in its metabolism in liver. For these reasons the status of free erythrose 4-phosphate monomer in liver and other tissues requires further investigation. It is of note that Dische & Igals (1961), Baxter *et al.* (1959), Severin & Stepanova (1973) and Blackmore *et al.* (1976) have all reported erratic and unexplainable behaviour of erythrose 4-phosphate in buffer and with different tissue extracts.

During the 17h incubation of rat liver enzyme preparation with [1-^{14}C]ribose 5-phosphate (see Fig. 2 of Williams *et al.*, 1978) there was a rapid and sustained decrease in the specific radioactivity of the [1-^{14}C]ribose 5-phosphate. It is most probable that this dilution of radioactivity of [1-^{14}C]ribose 5-phosphate was caused by the formation of unlabelled pentose 5-phosphate as a product of the transketolase-catalysed reaction involving glyceraldehyde 3-phosphate and D-*glycero*-D-*ido*-[4-^{14}C]octulose 8-phosphate (Scheme 3). Except for the lead given by the above phenomena, it is not presently possible to distinguish the contributions of either glyceraldehyde 3-phosphate (Scheme 3) or erythrose 4-phosphate (Scheme 1) to the reaction mechanism of the proposed new pathway. The involvement of either of these intermediates still allows the formation of the distinguishing [2-^{14}C]glucose 6-phosphate product (Williams *et al.*, 1978). The lack of precise information on the very small amounts of erythrose 4-phosphate (free aldehyde monomer) which may be present during the time course of reactions where ribose 5-phosphate was converted into hexose 6-phosphate (Williams *et al.*, 1978), together with the absence of information on possible biochemical roles of the three erythrose 4-phosphate dimers, the *gem*-diol of erythrose 4-phosphate, their interconversions and equilibria in cell extracts and reaction media *in vitro*, makes it difficult to resolve the nature of the aldehyde substrate in the final transketolase-catalysed reaction shown in Schemes 1 and 3.

The reaction sequence shown in Scheme 3 is also of interest, since it is consistent with the following definition (Utter, 1958): 'the pentose phosphate pathway may be viewed as a mechanism for the production of NADPH, pentose, erythrose 4-phosphate, glycolyl residues and under some circumstances as a mechanism for the conversion of pentoses to hexoses'. The reactions of Scheme 3 have a stoicheiometry which shows the conversion of 4mol of pentose 5-phosphate into 1mol of triose 3-phosphate, 1mol of erythrose 4-phosphate (which is in equilibrium with erythrose 4-phosphate dimers), 1mol of hexose 6-phosphate and 1mol of *altro*-heptulose 7-phosphate. All of these glycollyl units (except for erythrose 4-phosphate monomer) of varying carbon number were found as transient or accumulating intermediates during the time course of pentose 5-phosphate conversion into hexose 6-phosphate (Williams *et al.*, 1978).

It is also of note that equilibration of the [1,3-^{14}C]-dihydroxyacetone phosphate product of the reactions of either Scheme 1 or Scheme 3 with the triose phosphate pool would lead to the formation of [1,3,5-^{14}C]pentose 5-phosphate (Scheme 1) or [3,5-^{14}C]pentose 5-phosphate (Scheme 3). No movement

of ^{14}C from C-1 of [1-^{14}C]ribose 5-phosphate was ever observed during the 17h time course of the conversion of [1-^{14}C]ribose 5-phosphate into hexose 6-phosphate (Williams *et al.*, 1978).

The general outline and status of the pentose phosphate pathway of glucose metabolism was established from the pioneering work of Warburg, Dickens, Horecker, Racker, Kornberg and many others and the present view of the pathway is the integrated history of their contributions to this aspect of metabolism. The results of the past have certainly provided the firm experimental base from which it was possible for us to proceed to make the investigations reported here and to reach the conclusion that the pathway may be more complex than has been previously assumed. Although the proposed new pentose phosphate pathway is strongly supported by experimental evidence, we must, in the same spirit that Horecker *et al.* (1954) adopted, stipulate that the proposal is tentative and should be looked on as an extension of previous attempts to unravel some of the complexities of carbohydrate metabolism.

The work was supported by grants from the Australian Research Grants Committee, New South Wales State Cancer Council and the National Health and Medical Research Council. M. G. C. acknowledges the support of an Imperial Chemical Industries of Australia and New Zealand Postgraduate Fellowship. P. F. B. acknowledges the support of a Commonwealth Postgraduate Research Award, and J. F. W. and P. F. B. acknowledge the support of Fulbright Awards.

References

Ames, B. N. (1966) *Methods Enzymol.* **8**, 115–118
Ballou, C. E., Fisher, H. O. L. & MacDonald, D. L. (1955) *J. Am. Chem. Soc.* **77**, 5967–5970
Bartlett, G. R. & Bucolo, G. (1960) *Biochem. Biophys. Res. Commun.* **3**, 474–478
Bartlett, G. R. & Bucolo, G. (1968) *Biochim. Biophys. Acta* **156**, 240–253
Baxter, J. N., Perlin, A. S. & Simpson, F. J. (1959) *Can. J. Biochem. Physiol.* **37**, 199–209
Blackmore, P. F., Williams, J. F. & MacLeod, J. K. (1976) *FEBS Lett.* **64**, 222–226
Bonsignore, A., Pontremoli, S., Mangiarotti, G., De Flora, A. & Mangiarotti, M. (1962) *J. Biol. Chem.* **237**, 3597–3602
Clark, M. G., Williams, J. F. & Blackmore, P. F. (1971) *Biochem. J.* **125**, 381–384
Clark, M. G., Blackmore, P. F. & Williams, J. F. (1972) *J. Labelled Compd.* **8**, 637–652
Clark, M. G., Williams, J. F. & Blackmore, P. F. (1974) *Catal. Rev.* **9**, 35–77
Cohen, S. S. & McNair Scott, D. B. (1950) *Science* **111**, 543–544
Datta, A. G. & Racker, E. (1961) *J. Biol. Chem.* **236**, 617–623
Davies, D. D. (1961) in *Intermediary Metabolism in Plants*, p. 21, Cambridge University Press, Cambridge
Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research*, pp. 106–107, Clarendon Press, Oxford
Dickens, F. (1938) *Biochem. J.* **32**, 1626–1643
Dische, Z. & Igals, D. (1961) *Arch. Biochem. Biophys.* **93**, 201–210
Dische, Z., Shigeura, H. T. & Landsberg, E. (1960) *Arch. Biochem. Biophys.* **89**, 123–133
Dixon, M. (1953) *Biochem. J.* **55**, 170–171
Gibbs, M. & Horecker, B. L. (1954) *J. Biol. Chem.* **208**, 813–820
Glock, G. E. & McLean, P. (1954) *Biochem. J.* **56**, 171–175
Graham, D. & Smydzuk, J. (1965) *Anal. Biochem.* **11**, 246–255
Greenbaum, A. L., Gumaa, K. A. & McLean, P. (1971) *Arch. Biochem. Biophys.* **143**, 617–663
Gumaa, K. A. & McLean, P. (1968) *FEBS Lett.* **1**, 227–229
Gumaa, K. A. & McLean, P. (1969) *Biochem. J.* **115**, 1009–1029
Hohorst, H. J. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 134–138, Academic Press, New York
Horecker, B. L. (1957) *Methods Enzymol.* 3, 105–107
Horecker, B. L. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 111–113, Academic Press, New York
Horecker, B. L. & Mehler, A. H. (1955) *Annu. Rev. Biochem.* **24**, 207–274
Horecker, B. L. & Smyrniotis, P. Z. (1953) *J. Am. Chem. Soc.* **75**, 2021
Horecker, B. L. & Smyrniotis, P. Z. (1955) *J. Biol. Chem.* **212**, 811–825
Horecker, B. L., Gibbs, M., Klenow, H. & Smyrniotis, P. Z. (1954) *J. Biol. Chem.* **207**, 393–403
Hostetler, K. Y. & Landau, B. R. (1967) *Biochemistry* **6**, 2961–2964
Jones, J. K. N. & Sephton, H. H. (1960) *Can. J. Chem.* **38**, 753–760
Katz, J. & Wood, H. G. (1960) *J. Biol. Chem.* **235**, 2165–2177
Katz, J. & Wals, P. A. (1972) *Biochem. J.* **128**, 879–899
Kauffman, F. C. & Harkonen, M. H. A. (1977) *J. Neurochem.* **28**, 745–750
Klevstrand, R. & Nordal, A. (1950) *Acta. Chem. Scand.* **4**, 1320
Knox, W. E. (1972) *Enzyme Patterns in Fetal, Adult and Neoplastic Rat Tissues*, p. 302, S. Karger, Basel
Kornberg, H. L. & Racker, E. (1955) *Biochem. J.* **61**, iii–iv
Landau, B. R. & Katz, J. (1964) *J. Biol. Chem.* **239**, 697–704
Landau, B. R. & Katz, J. (1965) *Handb. Physiol. Sect.* 5 253–271
Landau, B. R., Bartsch, G. E., Katz, J. & Wood, H. G. (1964) *J. Biol. Chem.* **239**, 686–696
Levin, D. H. & Racker, E. (1959) *J. Biol. Chem.* **234**, 2532–2539
Ljungdahl, L., Wood, H. G., Racker, E. & Couri, D. (1961) *J. Biol. Chem.* **236**, 1622–1625
Mejbaum, W. (1939) *Hoppe-Seyler's Z. Physiol. Chem.* **258**, 117–120

Morton, R. K. (1958*a*) *Biochem. J.* **70**, 134–139
Morton, R. K. (1958*b*) *Biochem. J.* **70**, 139–150
Morton, R. K. (1958*c*) *Biochem. J.* **70**, 150–155
Novello, F. & McLean, P. (1968) *Biochem. J.* **107**, 775–791
Peterkofsky, A. & Racker, E. (1961) *Plant. Physiol.* **36**, 409–414
Pontremoli, S., Bonsignore, A., Grazi, E. & Horecker, B. L. (1960) *J. Biol. Chem.* **235**, 1881–1887
Racker, E. (1961*a*) *Enzymes 2nd Ed.* **5**, 402
Racker, E. (1961*b*) *Enzymes 2nd Ed.* **5**, 410
Racker, E. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 205–207, Academic Press, New York
Racker, E. & Schroeder, E. A. R. (1958) *Arch. Biochem. Biophys.* **74**, 326–344
Scott, D. B. M. & Cohen, S. S. (1951) *J. Biol. Chem.* **188**, 509–530
Segal, S. & Foley, J. B. (1959) *J. Clin. Invest.* **38**, 407–413
Severin, S. E. & Stepanova, N. G. (1973) *Biokhimiya* **38**, 583–588
Shafrir, E., Gutman, A., Gorin, E. & Orevi, M. (1970) in *Adipose Tissue Regulation and Metabolic Functions* (Levine, R. & Pfeiffer, E. F., eds.), pp. 130–135, Georg Thieme Verlag, Stuttgart and Academic Press, New York and London
Simpson, F. J., Perlin, A. S. & Sieben, A. S. (1966) *Methods Enzymol.* **9**, 35–38
Smith, I. (1958) *Chromatographic Techniques*, pp. 168–169, William Heinemann Medical Books, London
Srere, P., Cooper, J. R., Tabachnick, M. & Racker, E. (1958) *Arch. Biochem. Biophys.* **74**, 295–305
Stiller, M. (1962) *Annu. Rev. Plant Physiol.* **13**, 151–170
Stjernholm, R. L. & Noble, E. P. (1963) *Arch. Biochem. Biophys.* **100**, 200–204
Taussky, H. H. & Schorr, E. (1953) *J. Biol. Chem.* **202**, 675–685
Utter, M. F. (1958) *Annu. Rev. Biochem.* **27**, 245–284
Venkataraman, R. & Racker, E. (1961) *J. Biol. Chem.* **236**, 1876–1882
Vogel, A. I. (1967) *A Text-book of Practical Organic Chemistry*, 3rd edn., p. 87, Longmans-Green, London
Volk, W. A. (1959) *J. Biol. Chem.* **234**, 1931–1936
Volk, W. A. (1966) *Methods Enzymol.* **9**, 38–39
Williams, J. F., Rienits, K. G., Schofield, P. J. & Clark, M. G. (1971) *Biochem. J.* **123**, 923–943
Williams, J. F., Blackmore, P. F. & Power, P. A. (1974) *I.R.C.S. Med. Sci. Libr. Compend.* **2**, 1302
Williams, J. F., Clark, M. G. & Blackmore, P. F. (1978) *Biochem. J.* **176**, 241–256
Wood, H. G. & Katz, J. (1958) *J. Biol. Chem.* **233**, 1279–1282
Wood, H. G., Katz, J. & Landau, B. R. (1963) *Biochem. Z.* **338**, 809–847
Wood, T. (1968) *J. Chromatogr.* **35**, 352–361