Quantitative Measurement of the L-Type Pentose Phosphate Cycle with [2-¹⁴C]Glucose and [5-¹⁴C]Glucose in Isolated Hepatocytes

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(Received 30 October 1979)

1. Investigations of the mechanism of the non-oxidative segment of the pentose phosphate cycle in isolated hepatocytes by prediction–labelling studies following the metabolism of [2-¹⁴C]-, [5-¹⁴C]- and [4,5,6-¹⁴C]glucose are reported. The ¹⁴C distribution patterns in glucose 6-phosphate show that the reactions of the L-type pentose cycle are the exclusive form of the pentose pathway in hepatocytes. 2. Estimates of the quantitative contribution of the L-type pentose cycle to glucose metabolism have been made. The contribution of the L-type pentose cycle to the metabolism of glucose lies between 22 and 30% in isolated hepatocytes. 3. The distribution of ¹⁴C in the carbon atoms of glucose 6-phosphate following the metabolism of [4,5,6-¹⁴C]- and [2-¹⁴C]glucose indicate that gluconeogenesis from triose phosphate and non-oxidative formation of pentose 5-phosphate do not contribute significantly to randomization of ¹⁴C in isolated hepatocytes. The transaldolase exchange reaction between fructose 6-phosphate and glyceraldehyde 3-phosphate is very active in these cells.

It has not been possible to reach an agreement about the quantitative value of the pentose cycle in liver tissue due to the variability of a number of estimates (for a review see Katz, 1961). Previous estimates have ranged from values of 2–7% of total glucose metabolism based on studies *in vivo* (Hostetler & Landau, 1967; Marks & Feigelson, 1957) to 13–17% based on measurements of specific yields of ¹⁴C in CO₂ and lipid following the metabolism of [1-¹⁴C]- and [6-¹⁴C]glucose by isolated hepatocytes (Baquer *et al.*, 1973). This variability may be attributed to the use of theoretical expressions for the measurement of the pentose phosphate cycle, which have assumed that the reaction sequence of the non-oxidative segment of the pathway in liver was that proposed by Horecker *et al.* (1954) (see Katz & Wood, 1960, 1963). Recent work has shown that the reaction sequence for the non-oxidative segment of the pentose pathway is more extensive and complex than that of the classical pathway (Williams *et al.*, 1978). Theoretical expressions for estimating the contribution of the L-type pentose cycle to the metabolism of glucose have been derived [see the preceding paper (Longenecker & Williams, 1980)]. In practice these methods involve the application of [5-¹⁴C]- and [2-¹⁴C]glucose to the test tissue.

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

In the present experiments, the distribution of ¹⁴C in the carbon atoms of glucose 6-phosphate and lactate following the metabolism of [2-¹⁴C]-, [5-¹⁴C]- and [4,5,6-¹⁴C]glucose has been measured. The distribution of ¹⁴C in glucose 6-phosphate and lactate has been analysed in terms of the quantitative contribution of the L-type pentose cycle to the metabolism of glucose. The extent of other carbon-transferring mechanisms, including gluconeogenesis from triose phosphate (using [4,5,6-¹⁴C]glucose), transaldolase exchange reactions (using [2-¹⁴C]glucose; Ljungdahl *et al.*, 1961) and non-oxidative pentose 5-phosphate formation has been assessed.

Materials and Methods

Animals

Inbred male rats of the Buffalo strain, aged 8–10 weeks, were used in all experiments. The rats were fed *ad libitum* on a stock rat cube diet prior to isolation of hepatocytes.

Chemicals

All solvents were of the highest grade available from Ajax Chemicals (Sydney, Australia) or Hopkin and Williams (Chadwell Heath, Essex, U.K.). Collagenase (type CLS), batch no. 4196 CLS 48P003 was purchased from Worthington Biochemical Corp., Freehold, NJ, U.S.A. All enzymes,

substrates and coenzymes used were obtained from Boehringer or Sigma. All radioactive chemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. Other reagent chemicals were A.R. grade. Fatty-acid-free bovine serum albumin was obtained from Calbiochem.

Synthesis of [5-¹⁴C]glucose and [4,5,6-¹⁴C]glucose

[5-¹⁴C]Glucose was prepared from [2-¹⁴C]glycerol and [4,5,6-¹⁴C]glucose from [U-¹⁴C]glycerol using a modification of the method described by Ljungdahl *et al.* (1961). The reaction mixture (5.0 ml) contained: 100 μ mol of ATP, 200 μ mol of NAD⁺, 150 μ mol of fructose 6-phosphate, 1 mg of glycerokinase from *Candida mycoderma* (EC 2.7.1.30; Boehringer; 85 units/mg); 1 mg of glycerol 3-phosphate dehydrogenase from rabbit muscle (EC 1.1.1.8; Boehringer; 40 units/mg); 100 μ g of triose-phosphate isomerase from rabbit muscle (EC 5.3.1.1; Boehringer; 5000 units/mg); 1.3 mg of transaldolase from yeast (EC 2.2.1.2; Boehringer; 15 units/mg); 160 μ g of lactate dehydrogenase from rabbit muscle (EC 1.1.1.27; Boehringer; 550 units/mg); 200 μ mol of sodium pyruvate and 250 μ Ci of [2-¹⁴C]- or [U-¹⁴C]glycerol (25 Ci/mol). All substrates, cofactors and enzymes were made up in 100 mM-Tris/HCl (pH 7.8)/20 mM-MgCl₂. The reaction mixture was incubated for 2 h at 25°C before the addition of 50 units of yeast phosphoglucose isomerase (EC 5.3.1.9; Boehringer; 350 units/mg). The incubation was continued for 1 h and was then terminated by the addition of 5.0 ml of 0.6 M-HClO₄. The mixture was allowed to stand in ice for 30 min to complete the precipitation of protein. The protein was removed by centrifugation at 12000 g for 10 min in a Sorvall refrigerated centrifuge using the SS-34 rotor. The supernatant fluid was adjusted to pH 6.5 with KOH solution and allowed to stand at 0°C for 30 min. The precipitate of KClO₄ was removed by centrifugation as above, and the reaction mixture was placed on a column (1.5 cm $\times$ 30 cm) of Dowex 1 (X8; formate form; 200–400 mesh) (Bio-Rad, CA, U.S.A.) analytical grade resin followed by 50 ml of water. The column was eluted with a linear gradient, containing 500 ml of 2 M-formic acid in a mixing bottle and 4 M-formic acid in the reservoir, at a flow rate of 1 ml/min. Fractions (10 ml) were collected and the hexose 6-phosphate peak was identified using the anthrone reagent (Graham & Smydzuk, 1965) and the appropriate fractions were collected (total vol. 6 $\times$ 10 ml). The solution of ¹⁴C-labelled hexose 6-phosphates was concentrated under reduced pressure at 45°C using a Buchi rotary evaporator (Buchi Rotovapor R). The evaporated residue was then washed four times each with 100 ml of distilled water to remove the last traces of formic acid. The glucose 6-phosphate was separated from fructose 6-phosphate by descending paper chro-

matography using the GW3 solvent of Wood (1968). The glucose 6-phosphate was recovered from the paper by elution with water and concentrated to 5.0 ml under reduced pressure using a Buchi rotary evaporator. The glucose 6-phosphate solution was made up to a volume of 10 ml with a solution that was 100 mM-Tris/HCl (pH 10.4)/10 mM-MgCl₂ and dephosphorylated by the addition of 20 mg of alkaline phosphatase from calf intestine (EC 3.1.3.1; Sigma; 2 units/mg). The dephosphorylation incubation was maintained at 37°C for 3 h and was terminated by the addition of 10 ml of 0.6 M-HClO₄ and the precipitated protein was removed by centrifugation as above. The supernatant solution was de-ionized by passing it through tandem columns (1.2 cm $\times$ 20 cm) of Dowex 50 (H⁺ form) and Dowex 1 (X8; acetate form). The columns were washed with 100 ml of water and the appropriate fractions were pooled and evaporated to near dryness *in vacuo* at 40°C in a rotary film evaporator. The ¹⁴C-labelled glucose solution was evaporated to near dryness four times following each of 100 ml additions of distilled water to remove acetic acid. The solution was then concentrated to a final volume of 2.0 ml. Enzymic analysis of the glucose solution indicated that it was free from fructose. The specific radioactivity of the glucose was approximately 1.7 Ci/mol and the yield was 60 μ Ci. The distribution of ¹⁴C in the glucose was determined (Table 1).

Preparation and incubation of isolated hepatocytes

Isolated hepatocytes were prepared by the perfusion of the isolated rat liver with collagenase essentially as described by Berry (1974) except that hyaluronidase was omitted. Cells were judged to be greater than 90% viable by Trypan Blue exclusion.

The cells were incubated in Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, containing 5 mM-glucose, 0.2% fatty-acid-free-bovine serum albumin and 10.0 mM-Hepes at a concentration of approx. 150 mg wet wt. of cells/ml. The cells were preincubated in this buffer for 10 min prior to the addition of ¹⁴C-labelled glucose. The assay of glucose (Bergmeyer *et al.*, 1974) in the incubation medium at the end of the pre-incubation period indicated that the glucose concentration ranged between 6 and 8 mM and this concentration was maintained throughout the incubation period presumably due to glycogenolysis. It was not possible to estimate the utilization of glucose in these experiments by dilution of substrate level, since the fraction of isotope utilized was very small. Attempts to measure apparent utilization of glucose by measuring the incorporation of ¹⁴C into metabolic intermediates was also considered unreliable. In all experiments, incubations were carried out at 37°C for 1 h.

The incubations from which glucose 6-phosphate and/or lactate were isolated for degradation were carried out in a total volume of 10.0ml to which 10μCi of either [4,5,6-¹⁴C]-, [2-¹⁴C]- or [5-¹⁴C]-glucose was added. These incubations were terminated by the addition of 10.0ml of 0.6M-HClO₄ and the protein was removed by centrifugation. The hexose 6-phosphates were recovered by anion-exchange chromatography, dephosphorylated and purified as described above for the synthesis of [5-¹⁴C]glucose. To the glucose thus isolated, 1.0mmol of carrier glucose was added and the glucose was degraded carbon atom by carbon atom using *Leuconostoc mesenteroides* and appropriate chemical procedures (Williams *et al.*, 1971). An analytical control sample of glucose labelled with ¹⁴C in positions 1, 2 and 6 and of known isotopic composition was degraded simultaneously with the experimental samples.

Isolation and degradation of lactate

Lactate was isolated from the HClO₄ extract of cells that had been incubated with [2-¹⁴C]glucose by anion-exchange chromatography on Dowex 1 (X8; formate form; 200–400 mesh) using the same gradient as was described above for the ion-exchange chromatography of sugar phosphates. The lactate fraction was further purified by continuous liquid–liquid extraction with diethyl ether for 24h. This was followed by steam distillation and finally by paper chromatography using the solvent butan-1-ol/acetic acid/water (60:50:25, by vol.). The

lactate was chemically degraded (Williams *et al.*, 1971).

Results

Distribution of ¹⁴C in glucose 6-phosphate after the metabolism of [5-¹⁴C]glucose

The data of Table 1 show that 17.7% of the ¹⁴C in glucose 6-phosphate is incorporated into C-2 of that molecule after the metabolism of [5-¹⁴C]glucose. An estimate of the percentage contribution of the L-type pentose cycle can be made from the ratio of the specific radioactivities of C-2 and C-5 of glucose 6-phosphate using the expression:

$$PC = r_{H_{2,5}} / (1 - r_{H_{2,5}})$$

where *r*_{H_{2,5}} is the ratio C-2/C-5 (Longenecker & Williams, 1980). The C-2/C-5 ratio is (17.7–1.3)/70.8, or 0.23, and the resulting value of *PC* is 30%. As previously stated (Longenecker & Williams, 1980), this value represents the sum of the L-type pentose cycle contribution and ‘apparent’ metabolism as a consequence of ¹⁴C exchange occurring due to reversibility of either a transketolase–transaldolase series of reactions or reversal of the non-oxidative segment of the L-type cycle. The latter possibility seems unlikely, as previously discussed (Longenecker & Williams, 1980). The role of ¹⁴C exchange via the coupled transketolase–transaldolase reactions is treated in the Discussion. The data of Table 1 also show a significant incorporation of ¹⁴C into C-4 of hexose 6-phosphate. This distribu-

Table 1. Distribution of ¹⁴C in the ¹⁴C-labelled glucose substrates and in the glucose 6-phosphate isolated from liver cells after metabolism of [2-¹⁴C]-, [5-¹⁴C]- and [4,5,6-¹⁴C]glucose

Isolated hepatocytes were incubated for 1h at 37°C in the presence of 5mM-glucose labelled with ¹⁴C in C-2, C-5 or C-4, C-5 and C-6. Glucose 6-phosphate was isolated from HClO₄ extracts of the cells and was biologically and chemically degraded to determine the specific radioactivity of each carbon atom as described in the Materials and Methods section. The percentage recovery of ¹⁴C was determined by expressing the total ¹⁴C recovered (determined by the addition of the specific radioactivities of the six individual carbon atoms) as a fraction of the specific radioactivity obtained from the complete oxidation of the glucose moiety of glucose 6-phosphate by the method of Van Slyke & Folch (1940). A standard [1,2,6-¹⁴C]glucose sample accompanied the degradations described in this Table. Expected distribution of isotope in the standard was: C-1, 40%; C-2, 20%; C-6, 40%; distribution found; C-1, 37%; C-2, 19%; C-3, 0%; C-4, 1%; C-5, 2%; C-6, 42%. The results are the mean of the number of determinations in parentheses. The S.E.M. is shown for values based on more than three determinations.

Percentage of ¹⁴ C found						
Carbon atom	Substrates			Glucose 6-phosphate from incubations of cells with		
	[2- ¹⁴ C]- Glucose (2)	[5- ¹⁴ C]- Glucose (2)	[4,5,6- ¹⁴ C]- Glucose (1)	[4,5,6- ¹⁴ C]- Glucose (2)	[2- ¹⁴ C]- Glucose (7)	[5- ¹⁴ C]- Glucose (4)
1	0	0	1.2	2.0	2.5 ± 0.8	0.6 ± 0.2
2	96.5	1.3	1.6	12.1	66.8 ± 5.7	17.7 ± 2.7
3	0.4	0.9	1.7	2.7	4.2 ± 0.9	1.9 ± 0.2
4	1.2	2.1	33.0	30.0	1.8 ± 0.4	11.5 ± 2.7
5	0.6	94.0	31.4	24.3	21.8 ± 4.0	70.8 ± 5.6
6	1.0	1.7	31.0	28.7	2.9 ± 0.9	1.4 ± 0.3
Recovery	87	93	87	96	84	87

tion of ^{14}C cannot be explained by any known ^{14}C transfer reactions.

Distribution of ^{14}C in glucose 6-phosphate following the metabolism of $[2-^{14}\text{C}]$ - and $[4,5,6-^{14}\text{C}]$ glucose

Because of the potential complexity of glucose metabolism in liver, it was considered necessary to investigate the possible operation of two sets of reactions that theoretically could influence the distribution of isotope between C-2 and C-5 of hexose 6-phosphate. The two reactions are (1) the synthesis of hexose 6-phosphate from labelled triose phosphate, catalysed by aldolase and fructose biphosphatase (EC 3.1.3.11) and (2) the transaldolase reaction that exchanges C-4, C-5 and C-6 of fructose 6-phosphate with glyceraldehyde 3-phosphate (Ljungdahl *et al.*, 1961). To estimate the contribution of these reactions, which could act to qualify the measurements of the L-type pentose cycle (Longenecker & Williams, 1980), cells were incubated with either $[2-^{14}\text{C}]$ - or $[4,5,6-^{14}\text{C}]$ glucose and the distribution of ^{14}C in the carbon atoms of glucose 6-phosphate was determined as shown in Table 1.

The results indicate that gluconeogenesis from ^{14}C -labelled triose phosphate in isolated hepatocytes does not significantly alter the ^{14}C distribution in the carbon atoms of glucose 6-phosphate, since very little ^{14}C is found in positions 1 or 3. For example, the net increase in the percentage of ^{14}C in C-3 of hexose 6-phosphate is only 1%, which indicates that the maximum redistribution of ^{14}C from $[5-^{14}\text{C}]$ -glucose into C-2 of hexose 6-phosphate would be approximately 3%. If this value is used to correct for the effect of gluconeogenesis, the C-2/C-5 ratio becomes $(17.1 - 1.3 - 3.0)/(70.8 + 3.0) = 0.18$ and the value of PC is 22%. It should be noted, however, that a redistribution of 1% of ^{14}C is subject to considerable experimental error. It is of note that only C-2 of glucose 6-phosphate shows significant incorporation of ^{14}C (10.5%) and that the greatest loss of ^{14}C is from C-5 (7.1%). The isotope labelling pattern is consistent with the predicted redistribution of ^{14}C via the L-type pentose cycle. The C-2/C-5 ratio after the metabolism of $[4,5,6-^{14}\text{C}]$ -glucose is higher than the ratio following the metabolism of $[5-^{14}\text{C}]$ glucose. This difference may be attributed to the errors inherent in the determination of the specific radioactivities of the carbon atoms of glucose and the fact that very small changes in the percentage of ^{14}C in C-2 result in large changes in the ratio. Since gluconeogenesis does not influence the ^{14}C distribution in glucose 6-phosphate, it is concluded that the relocation of 21.8% of the isotope from $[2-^{14}\text{C}]$ glucose into C-5 of glucose 6-phosphate (Table 1) results from the transaldolase exchange reaction.

The extent of ^{14}C incorporation into C-5 of

glucose 6-phosphate following the metabolism of $[2-^{14}\text{C}]$ glucose indicates that the transaldolase exchange reaction is very active under these experimental conditions. If the specific radioactivity of the triose phosphate pool is assumed to be one-half that of the glucose substrate, it can be shown that isotopic equilibrium is approached between C-4, C-5 and C-6 of hexose 6-phosphate and the triose phosphates. Although the extent of the transaldolase exchange reaction is great, and will act to dilute the specific radioactivity of C-5 of hexose 6-phosphate, this dilution will have no effect on the C-2/C-5 ratio after the metabolism of $[5-^{14}\text{C}]$ glucose and therefore will not alter estimates of the L-type pentose cycle (for a discussion of the transaldolase exchange reaction see the preceding paper: Longenecker & Williams, 1980).

The distribution of ^{14}C in the carbon atoms of glucose 6-phosphate following the metabolism of $[2-^{14}\text{C}]$ glucose may also be used to calculate any contribution of the F-type pentose pathway, based on the theoretical expression derived by Katz & Wood (1960). This calculation requires a measurement of the specific radioactivities of C-1, C-2 and C-3 of hexose 6-phosphate after $[2-^{14}\text{C}]$ glucose metabolism. The data of Table 1 show that very little ^{14}C is distributed into C-1 or C-3 from $[2-^{14}\text{C}]$ -glucose, and calculations using the expression of Katz & Wood (1960) result in values of 2.0 and 3.1% for the percentage of glucose metabolized via the F-type pentose pathway in liver. These values are in good agreement with those reported by Hostetler & Landau (1967) using rat liver *in vivo*, and indicate that there is an insignificant level of the F-type pentose cycle in liver.

Distribution of ^{14}C in lactate after the metabolism of $[2-^{14}\text{C}]$ glucose

The reaction sequence of the non-oxidative segment of the L-type pentose phosphate pathway results in a unique distribution of ^{14}C in triose phosphate after the metabolism of $[2-^{14}\text{C}]$ glucose. This redistribution results in the ratio of the specific radioactivity of C-1 to C-3 of triose phosphate being unity for any contribution of the L-type pentose cycle to glucose metabolism. An expression has been derived for estimating the percentage contribution of the L-type cycle to the metabolism of glucose relative to glycolysis, based upon the ratio of the specific radioactivities of C-1 or C-3 to C-2 of triose phosphate or its derivatives (Longenecker & Williams, 1980). In practice, the relatively small pool of triose phosphates makes the isolation and degradation of triose phosphate difficult, and therefore a derivative of triose phosphates such as lactate or glycerol is chosen. In liver the more accessible intermediate is lactate. In choosing lactate as the triose phosphate derivative, it is assumed that the

Table 2. *Distribution of ^{14}C in the carbon atoms of lactate formed by the metabolism of $[2-^{14}\text{C}]$ glucose by isolated hepatocytes*

Isolated hepatocytes were incubated for 1 h at 37°C in the presence of 5 mM $[2-^{14}\text{C}]$ glucose. Lactate was isolated and purified from HClO_4 extracts of the cells and was chemically degraded to determine the specific radioactivity of each carbon atom as described in the Materials and Methods section. The percentage recovery of label was determined by expressing the total label recovered (i.e. the addition of the specific radioactivities of the three carbon atoms) as a fraction of the specific radioactivity obtained by complete oxidation of the lactate by the method of Van Slyke & Folch (1940). The results are the mean $\pm$ S.E.M. of seven determinations.

Carbon atom	Percentage of radioactivity	Ratio of specific radioactivities
1	9.5 ± 0.9	$\text{C1/C3} = 0.52$
2	72.1 ± 2.3	$\text{C1/C2} = 0.13$
3	18.4 ± 1.6	$\text{C3/C2} = 0.26$
Recovery	84	

^{14}C distribution in the carbon atoms of lactate reflects the distribution of ^{14}C in triose phosphates formed via the L-type pentose cycle and glycolysis only, and that no other carbon-transferring reactions such as CO_2 fixation or recycling in the tricarboxylic acid cycle occur.

The results of Table 2 show the distribution of ^{14}C in the carbon atoms of lactate following the metabolism of glucose by isolated hepatocytes. The C-1/C-3 ratio is not unity. Since the data of Table 2 are not consistent with the theoretical predictions on which the expression for quantitatively estimating the L-type pentose cycle were derived, the C-1/C-2 ratio in lactate cannot be used to estimate the contribution of the pentose cycle to the metabolism of glucose by isolated hepatocytes.

Discussion

Mechanism of the non-oxidative segment of the pentose cycle in isolated hepatocytes

The work reported here was designed in part as prediction-labelling experiments. The sequence of the reactions of the non-oxidative segment of the L-type pentose cycle proposed by Williams *et al.* (1978) predicts the redistribution of isotope from $[5-^{14}\text{C}]$ glucose into C-2 of hexose 6-phosphate (Longenecker & Williams, 1980). The reaction sequence of the F-type pathway would predict no randomization of ^{14}C from $[5-^{14}\text{C}]$ glucose. The data of Table 1 are entirely consistent with the operation of the L-type pentose phosphate pathway in liver, since a net increase of 16.4% of the ^{14}C in glucose

6-phosphate recovered from cells incubated with $[5-^{14}\text{C}]$ glucose was located in C-2. The only other known mechanism for relocating ^{14}C from $[5-^{14}\text{C}]$ glucose into C-2 of glucose 6-phosphate is gluconeogenesis from $[2-^{14}\text{C}]$ triose phosphates. The extent of these reactions appears to be quite small, since the metabolism of $[4,5,6-^{14}\text{C}]$ glucose yielded a net increase of only 0.8 and 1.0% of ^{14}C in C-1 and C-3 of glucose 6-phosphate.

^{14}C distribution in lactate

The data of Table 2 may be interpreted in two ways. One explanation involves the possibility that the F-type and not the L-type mechanism of the cycle operates in isolated hepatocytes. The other is that labelling of lactate does not reflect the ^{14}C -labelling pattern in triose phosphates formed via the pentose cycle and glycolysis in liver, rather that the ^{14}C distribution is the consequence of the further metabolism of triose phosphate derivatives. The weight of experimental evidence favours the latter interpretation.

With respect to the mechanism of the non-oxidative segment of the pentose pathway in hepatocytes, randomization of ^{14}C following the metabolism of $[2-^{14}\text{C}]$ glucose via the F-type pentose cycle should introduce about twice as much ^{14}C into C-1 of glucose 6-phosphate as into C-3, and therefore the C-1/C-3 ratio in lactate should be 0.5 (Katz & Wood, 1960). Although the C-1/C-3 ratio shown in Table 2 is close to 0.5, the corresponding C-3/C-1 and C-6/C-4 ratios in glucose 6-phosphate (Table 1) are not consistent with the ratio found in lactate. It is a condition of the F-type pentose cycle mechanism that all ^{14}C distribution in triose phosphate arises from glycolysis of reformed hexose 6-phosphate. Therefore, the lack of agreement between the ^{14}C distribution in glucose 6-phosphate and the carbon atoms of lactate indicates that lactate does not reflect the ^{14}C distribution pattern in hexose 6-phosphate formed via the pentose cycle. Furthermore, the distribution of ^{14}C in glucose 6-phosphate after $[5-^{14}\text{C}]$ glucose metabolism is entirely consistent with the operation of the L-type pentose cycle. The only tissue in which the operation of the F-type pathway has been demonstrated is rat adipose tissue (Williams *et al.*, 1974). Further evidence supporting the view that there is little value in isolating and degrading metabolic intermediates that are distant from the phosphorylated intermediates of the pentose cycle is illustrated by the experiments of Katz *et al.* (1966), which showed that the ^{14}C distribution in the carbon atoms of lactate after the metabolism of $[2-^{14}\text{C}]$ glucose by rat epididymal fat-pads could not be used to estimate the contribution of the pentose cycle, since the C-1/C-3 ratio in the carbon atoms of lactate was twice that found in glycerol.

It may be concluded that the ^{14}C distribution in the carbon atoms of lactate is not a reflection of the ^{14}C distribution in triose phosphate formed as a consequence of the pentose cycle and the Embden-Meyerhof pathway. This interpretation is strengthened by the data of Grunnet & Katz (1978), Mullhofer *et al.* (1977) and Friedmann *et al.* (1971) who reported the redistribution of ^{14}C into metabolic intermediates from $[1-^{14}\text{C}]$ - and $[2-^{14}\text{C}]\text{lactate}$ metabolism by liver consistent with the operation of a futile cycle involving the following reaction sequence: lactate $\rightarrow$ pyruvate $\rightarrow$ oxaloacetate $\rightarrow$ malate $\rightarrow$ fumarate $\rightarrow$ malate $\rightarrow$ oxaloacetate $\rightarrow$ phosphoenolpyruvate $\rightarrow$ pyruvate $\rightarrow$ lactate. The effect of such reactions on the ^{14}C distribution from $[2-^{14}\text{C}]\text{lactate}$ would be to increase the amount of ^{14}C in C-3 of lactate, and thus decrease the C-1/C-3 ratio, which is consistent with the data of Table 2.

Quantitative estimates of the pentose cycle in isolated hepatocytes

It has been established (Longenecker & Williams, 1980) that the value for the percentage contribution of the pentose cycle calculated from the C-2/C-5 ratio in glucose 6-phosphate is the sum of the oxidative metabolism and the contribution of ^{14}C exchange reactions. Calculations based on ^{14}C distribution can be misleading, since they do not necessarily measure net flow of carbon; instead they frequently measure incorporation of ^{14}C through exchange reactions. In the extreme case, the ^{14}C distribution in glucose 6-phosphate after the metabolism of $[5-^{14}\text{C}]\text{glucose}$ shown in Table 1 could arise entirely from the coupled transketolase-transaldolase reactions, or by reversal of the reactions of the L-type pentose cycle with no glucose-carbon flux through the oxidative segment of the pentose cycle.

A qualitative estimate of the extent of ^{14}C distribution due to isotope exchange via transketolase-transaldolase reactions can be made from the analysis of the distribution of ^{14}C in the carbon atoms of glucose 6-phosphate after the metabolism of $[2-^{14}\text{C}]\text{glucose}$. Non-oxidative formation of pentose 5-phosphate via the coupled transketolase-transaldolase exchange mechanism from $[2-^{14}\text{C}]\text{glucose}$ yields $[2-^{14}\text{C}]\text{pentose 5-phosphate}$ (Katz & Rognstad, 1967). When $[2-^{14}\text{C}]\text{pentose 5-phosphate}$ is metabolized via the L-type pentose cycle, the ^{14}C is redistributed into C-3 of glucose 6-phosphate. Examination of the data in Table 1 shows very little incorporation of ^{14}C into C-3 of glucose 6-phosphate from $[2-^{14}\text{C}]\text{glucose}$. It is concluded that non-oxidative formation of pentose 5-phosphate via the coupled transketolase-transaldolase reactions does not contribute to any great extent to the redistribution of ^{14}C in glucose 6-phosphate.

It is a theoretical condition of the method for

measuring the L-type pathway that the triose phosphates produced by reactions F and G do not mix with the pool of triose phosphates produced by the Embden-Meyerhof pathway and by reaction H (see Fig. 2 of the preceding paper: Longenecker & Williams, 1980). If they equilibrated completely with this pool, a C-2/C-5 ratio of 0.25 would represent 100% L-type pentose cycle. However, mixing would cause incorporation of ^{14}C from $[2-^{14}\text{C}]\text{glucose}$ into C-1 of glucose 6-phosphate. The data of Table 1 show that little such labelling was found, so that the theoretical condition was substantially met.

The redistribution of 13–17% of ^{14}C from $[5-^{14}\text{C}]\text{glucose}$ into C-2 of hexose 6-phosphate is largely due to the reactions of the L-type pentose cycle. The quantitative contribution of the L-type pentose cycle to the metabolism of glucose lies between 22 and 30% based upon the ratio of the specific radioactivities of C-2 and C-5 of glucose 6-phosphate. The contribution of ^{14}C exchange reactions does not appear to be large, based on the redistribution of ^{14}C in C-3 of hexose 6-phosphate following the metabolism of $[2-^{14}\text{C}]\text{glucose}$. Theoretically, these values may represent an underestimate, since it has been assumed that there is no isotopic equilibration of the pools of triose phosphate but complete isotopic equilibration of glucose 6-phosphate with fructose 6-phosphate. The values reported here are considerably higher than any previously reported for liver and show that glucose carbon flux through the L-type pentose cycle is a major component of glucose metabolism in liver. This finding is consistent with the high levels of pentose pathway enzymes found in liver (Novello & McLean, 1968) relative to the activities of enzymes that catalyse glucose catabolism by 'alternate' pathways.

This work was supported by grants from the Australian Research Grants Committee.

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