THE MECHANISM OF THE NON-OXIDATIVE SEGMENT OF THE PENTOSE CYCLE IN THE LIVER

Robert Rognstad and Joseph Katz

Cedars-Sinai Medical Research Institute
4751 Fountain Avenue
Los Angeles, California 90029

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Summary: [1-^{14}C] Xylitol was used as substrate for isolated liver parenchymal cells from fasted rats or hamsters, and the glucose formed was isolated and degraded. Over 90% of the total radioactivity was in carbons 1 and 3, and the ratio of specific activity of C-1 to that of C-3 was only slightly under two, the expected ratio for the classical mechanism of the non-oxidative pentose phosphate pathway.

The generally accepted mechanism of the non-oxidative segment of the pentose cycle was first proposed by Horecker et al (1). Recently Williams and Clark (2) have presented evidence from which they have concluded that "the accepted metabolic sequence for the pentose phosphate cycle....is shown to be incorrect." They have repeated and enlarged upon the original isotopic experiments of Horecker et al (1), incubating [1-^{14}C] ribose 5 phosphate with liver extracts for varying time periods. While the results of their long term (up to 17 hours) experiments are similar to the original results of Horecker et al (1), the data found at short incubation periods (from 1 to 30 min) are not at all what would be expected by the classical non-oxidative pentose phosphate pathway.

A number of the methods for the estimation of the rate of the pentose cycle in the intact cell (3, 4, 5, 6) have been based upon the formulation of the cycle given by Horecker et al (1). Thus we felt it important to attempt to reexamine the basis of this long accepted scheme.

METHODS: Rats or hamsters were fasted for 24 hours prior to preparation of liver cells essentially by the method of Berry and Friend (7). Liver cells (from 50 to 200 mg wet weight) were incubated at 37° in 25 ml glass Erlenmeyer flasks in 2 ml of Krebs-Henseleit (8) buffer containing 20 mM [1-^{14}C] xylitol. The incubations were stopped by addition of 0.5 ml of 1N H_2SO_4 and the cells and medium made to a volume of 10 ml and centrifuged. Of this, 9 ml was put on a column (1 cm x 3 cm) of Amberlite CG-120 (H^+) on top of a column (1 cm x 6 cm) of Dowex 1 x 8 (acetate) and the columns were washed with water until 30 ml was collected. To this were added triethanolamine-HCl buffer, pH 7.5 (1000 μmoles), NaOH (500 μmoles), ATP (25 μmoles), $MgCl_2$ (50 μmoles) and hexokinase (4 units, Sigma type C-302, pretested for absence of glucose-6P isomerase). After 3 hours, assay (9) for free glucose was usually negative. The mixture was put on ion exchange columns (as above), which were washed with an additional 30 ml of water. Glucose-6-phosphate was eluted from the Dowex-1 column with 4N formic acid, dried in a stream of air, and treated with 1 mg (2 units) of acid phosphatase in 3 ml of .33 M Na acetate buffer, pH 5.0, for 24 hours at room temperature. The mixture was again put through small cation and anion exchange columns and the neutral effluent dried. The glucose was degraded by the procedure of Schmidt et al (10).

[1-^{14}C] Xylitol was prepared by reduction of [1-^{14}C] D-xylose (Biochemical and Nuclear Corporation, Burbank, California) with excess $NaBH_4$ for 8 hours. The reaction mixture was applied to a 1 cm x 20 cm column of Dowex 1 x 8 (borate form, 100-200 mesh). [1-^{14}C] Lyxitol (from a [1-^{14}C] D-lyxose impurity in the commercial [1-^{14}C] D-xylose) was eluted with .025 M Na tetraborate, after which [1-^{14}C] xylitol was eluted with .035 M Na tetraborate. The

^{14}C peaks were identical to the ^{3}H peaks obtained on a similar column to which was applied the reaction products of the incubation of D-xylulose with [^{3}H] $NaBH_4$. The active fractions were put through an Amberlite CG-120 (H^+) column to remove Na^+, dried in a rotary vacuum evaporator, and redried several times after addition of methanol, to remove boric acid. The [1-^{14}C] xylitol was further purified by descending chromatography on Whatman 3 mm paper, using n-butanol/acetic acid/water (4/1/2, by volume).

RESULTS AND DISCUSSION: We feel that the most reliable approach to testing a proposed metabolic pathway with experimental isotopic data can be made when the flux in the pathway is high. In this way extraneous exchange reactions which could lead to errors in interpretation will be minimized. Also the use of the intact cell rather than cellular homogenates or extracts has advantages which are obvious. Thus we have chosen to reexamine the nature of the non-oxidative pentose phosphate pathway in the liver by using xylitol as the gluconeogenic substrate (since possibly only fructose is consistently superior to xylitol in rate of conversion to glucose), and by using enzymically prepared isolated liver parenchymal cells in which rates of gluconeogenesis are very similar to those obtained *in vivo*. The pathway of xylitol conversion to glucose, following oxidation to xylulose and activation to xylulose-5P, is expected to follow the non-oxidative pentose cycle to hexose phosphate and triose phosphate.

Since Williams and Clark (2) have obtained markedly varying isotopic distribution patterns in hexose-P (and in other intermediates) with time of incubation, we have likewise carried out time course studies in our experiments. Table I shows the patterns of ^{14}C distribution in the glucose formed from [1-^{14}C] xylitol added to liver cells. In the time course experiment

TABLE I. DISTRIBUTION OF ^{14}C IN GLUCOSE FOLLOWING METABOLISM OF [1-^{14}C] XYLITOL BY ISOLATED LIVER CELLS.

Rat or hamster liver cells from 24 hour fasted animals were incubated with 20 mM [1-^{14}C] xylitol (1 μC, except in the 2.5 and 5 min incubations of experiment 1 where 3 μC was used) in 2 ml of Krebs-Henseleit buffer. Rates of gluconeogenesis were 100, 80 and 60 μmoles/gm wet weight/hour in experiments 1, 2 and 3 respectively. Glucose was isolated and degraded as described in the methods section.

Expt. No.	Source of Liver	Amount of Liver Cells (mg/dry/wt)	Incubation Time (Minutes)	[^{14}C] Xylitol to Glucose (% Added ^{14}C)	Distribution of ^{14}C in Glucose % of Total Activity C-1	C-2	C-3	C-4, 5, 6
1	Hamster	50	2.5	2.8	61.8	1.5	32.7	4.0
			5	5.3	62.3	1.4	33.4	2.9
			15	18.0	62.1	1.4	34.2	2.3
			30	35.1	62.7	1.4	33.7	2.2
2	Hamster	13	45	9.5	61.0	1.9	31.2	5.9
3	Rat	25	45	13.8	60.2	1.9	31.2	6.7

with hamster liver cells the rate of gluconeogenesis was very nearly linear over the 30 min period and averaged about 100 μmoles glucose per gm wet weight of cells per hour. The distribution of ^{14}C in glucose formed at all times including the earliest (2.5 min) was very near to that expected from the generally accepted formulation of the pentose cycle, in which the amount of radioactivity in C-1 of glucose should be twice that in C-3. The slight deviation (in all experiments) from the expected classical pattern may be attributed to a small amount of a complete pentose cycle (oxidative and non-oxidative pathways) in addition to the major flux in the non-oxidative pathway. Evidence for some oxidative pathway is from the small amount of randomization of ^{14}C into C-2 of glucose. The small amount of radioactivity in carbons 4, 5, and 6 of glucose may be attributed to some phosphofructokinase activity, forming a degree of futile cycling between fructose diphosphate and fructose-6-phosphate due to simultaneous action of fructose-diphosphatase (high rate) and phosphofructokinase (low rate). We have shown previously (11) that during active gluconeogenesis in fasted liver cells, with [1-^{14}C] galactose added at tracer levels, the glucose formed shows a small degree of labelling in C-6, presumably due to a phosphofructokinase back reaction, followed by partial isotopic equilibration of the triose phosphates in the aldolase-triose phosphate isomerase system.

The results of our experiments are thus fully in accord with the widely accepted mechanism of the pentose cycle. On the other hand Williams and Clark and coworkers have presented an impressive amount of data on specific activities and ^{14}C distributions in products and intermediates of the pentose phosphate pathway which are at variance with the classical scheme (2, 12). How-

ever little of their data was obtained with active intact cell preparations. Williams et al (13, 14) have employed a clamped (and therefore ischemic) rabbit liver in situ, in which the ATP/ADP ratio rapidly decreased and thus in which little biosynthesis would be expected. Most of their data have been obtained on cell extracts. In their repeat (2) of the original Horecker et al (1) experiment using [1-^{14}C] ribose 5 phosphate and a rat liver acetone powder extract, at short times the major amount of radioactivity was in carbons 2 and 6 of the glucose-6-phosphate isolated. The labelling on C-2 was attributed to a pathway involving epimerization to [1-^{14}C] arabinose 5P, an aldolase reaction to [4-^{14}C] octulose 1,8 diphosphate with subsequent conversion to [4-^{14}C] octulose 8 phosphate, followed by transketolase reaction of this with erythrose 4 phosphate to form [2-^{14}C] glucose-6-phosphate. The labelling on C-6 of glucose-6-phosphate was attributed to a pathway involving aldolase cleavage of [1, 3-^{14}C] dihydroxyacetone phosphate, followed by isomerization and a transaldolase exchange reaction. Arabinose 5P accumulated during the early periods of incubation, and they demonstrated that arabinose 5P is an inhibitor of transaldolase. They suggested that arabinose 5P thus may play a role in the inhibition of the Horecker pathway (involving the sequence of reactions transketolase, transaldolase, transketolase) in the early phase of these experiments. While all these explanations may be correct in the interpretation of the liver extract experiments, the question remains as to their significance in the operation of the pentose cycle in the intact liver cell.

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REFERENCES

1. Horecker, B.L., Gibbs, M., Klenow, H. and Smyrniotis, P.Z. (1954) J. Biol. Chem. 207, 393-403.
2. Williams, J.F. and Clark, M.G. (1971) Search 2, 80-88.
3. Katz, J. and Wood, H.G. (1958) J. Biol. Chem. 235, 2165-2177.
4. Landau, B.R., Bartsch, G.E., Katz, J. and Wood, H.G. (1964) J. Biol. Chem. 239, 686-696.
5. Katz, J. and Rognstad, R. (1967) Biochemistry 6, 2227-2247.
6. Katz, J. and Wals, P.A. (1972) Biochem. J. 128, 879-899.
7. Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506-520.
8. Krebs, H.A. and Henseleit, K. (1932) Z. Physiol. Chem. 210, 33-66.
9. Rognstad, R. and Clark, D.G. (1974) Arch. Biochem. Biophys. 161, 638-646.
10. Schmidt, K., Genovese, J. and Katz, J. (1970) Anal. Biochem. 34, 170-179.
11. Rognstad, R., Clark, D.G. and Katz, J. (1973) Biochem. Biophys. Res. Comm. 54, 1149-1156.
12. Clark, M.G., Williams, J.F. and Blackmore, P.F. (1972) Search 3, 87-88.
13. Williams, J.F., Rienits, K.G. and Clark, M.G. (1971) Biochem. J. 123, 915-922.
14. Williams, J.F., Rienits, K.G. and Clark, M.G. (1971) Biochem. J. 123, 923-943.