# EFFECT OF OXAMATE, PYRUVATE, NICOTINAMIDE AND STREPTOZOTOCIN ON THE PENTOSE PHOSPHATE PATHWAY INTERMEDIATES IN ASCITES TUMOUR CELLS

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Measurement of the transient and steady-state concentrations of intermediates of the pentose phosphate pathway have indicated that there is a rapid synthesis of metabolites of this pathway on addition of glucose to Krebs ascites cells in vitro, as was shown by Gumaa, McLean and Bennette (1). It has been shown that the rate of reoxidation of NADPH is a critical factor in the regulation of the direct oxidative reactions of the pentose phosphate cycle in many tissues including ascites tumour cells (2,3) and there is a considerable amount of evidence that pyruvate is an important electron acceptor in the reoxidation of NADPH by ascites cells in vitro (3).

In order to gain further insight into the control of the direct oxidative and non-oxidative reactions of the pentose phosphate pathway, it seemed of interest to study the effect on metabolite levels of some compounds modifying the redox state and concentration of the nicotinamide nucleotides. Pyruvate and oxamate were used to alter the redox state of $NAD^+$ and $NADP^+$, pyruvate by increasing the rate of reoxidation of the nicotinamide nucleotides in a coupled reaction with lactic dehydrogenase (3) and oxamate by inhibiting the same dehydrogenase (4).

The effect of treatment of mice bearing ascites cells with streptozotocin or nicotinamide, which lowers and raises the $NAD^+$ content of the ascites cells respectively (5), on the pentose phosphate pathway intermediates were also studied, and from the work of Clark, Greenbaum and McLean (6) it was antici-

pated that these two compounds might also influence the $NADP^+$ content of the cells. In addition, measurements were made of the conversion of specifically-labelled glucose into $^{14}CO_2$ in order to gain an overall picture of the influence of these treatments on the flux of glucose through the alternative pathways of metabolism.

It was found that pyruvate raised the concentration of metabolites of the pentose phosphate pathway and oxamate lowered them. Streptozotocin lowered the 6-phosphogluconate and raised sedoheptulose 7-phosphate and erythrose 4-phosphate, showing a converse picture to nicotinamide treatment which raised the 6-PG content of the ascites cells. The results are discussed in relation to the control of the direct oxidative steps of the pentose phosphate pathway by $NADP^+$ availability and to the route of synthesis of pentose phosphates by the direct oxidative or the non-oxidative reactions of the cycle, both of which seem to participate with a predominance of the latter route.

## MATERIALS AND METHODS

Purified enzymes and substrates used in the assay of the metabolites were all purchased from Boehringer & Soehne, Mannheim, Germany, with the following exceptions: transaldolase was a gift from Dr. B.L. Horecker; the mixture of ribulose 5-phosphate epimerase and ribose 5-phosphate isomerase was prepared as in (7); transketolase was prepared by the method of Simpson (8) with the modifications cited in (1). Oxamic acid was from BDH and was neutralised with KOH on use; Nicotinamide was from BDH and was freshly dissolved in 0.9% NaCl. Streptozotocin, a gift from Dr. A. Beloff Chain, was freshly dissolved in 0.005 M citrate buffer, pH 4.0. $^{14}C$-labelled glucose was purchased from the Radiochemical Centre, Amersham, England.

Krebs 2 ascites cells were harvested 7 days after implantation and were then processed as in (1). Where nicotinamide or streptozotocin was used, the mice bearing the tumour cells were injected I/P with 500 mg/kg body wt. or 200 mg/kg body wt. respectively (5) and the cells processed 4 hours later.

Incubations were carried out at 37° with shaking in flasks fitted with centre wells and rubber caps. Where used, potassium oxamate was added to a final concentration of 0.08 M (4,9) and pyruvate to 0.01 M (3). Flasks were gassed with 100% oxygen for 10 min. and glucose was then injected through

the caps to a final concentration of 10 mM and 0.5 μC. Incubations continued for 10 min. and were terminated by deproteinisation as in (1). $^{14}CO_2$ was collected into 1 ml of hyamine solution.

Metabolites were assayed by standard methods described by Bergmeyer (10) and the pentose phosphate pathway intermediates were assayed as in (1). Ribose, ribulose and xylulose 5-phosphates were assayed together as pentose phosphates because of the difficulty of separating the necessary enzymes.

All results are expressed as percentages of control values obtained by incubating the tumour cells with 10 mM glucose.

## RESULTS AND DISCUSSION

The effect of pyruvate addition to ascites cells metabolising glucose on the intermediates of the pentose phosphate pathway is shown in Figure I.

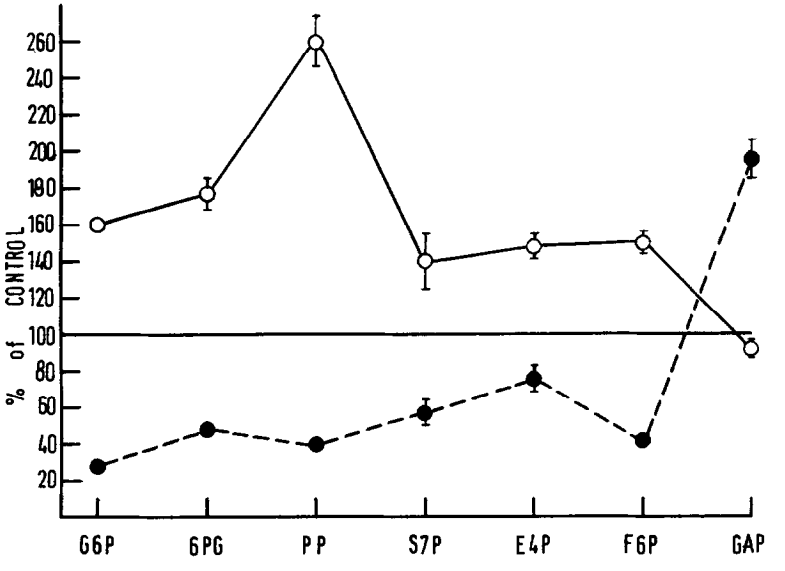


Figure I. Effect of pyruvate and oxamate on levels of intermediates of the pentose phosphate pathway in Krebs ascites cells.

Results are expressed as percentages of control values ± S.E.M. Where no S.E.M. is given, it is less than 4%. Control values have an S.E.M. less than 4% and are not shown.

Open circles represent pyruvate effect and closed circles oxamate effect. Each point represents the mean of at least six observations.

G-6-P = glucose 6-phosphate; 6-PG = 6-phosphogluconate; P.P. = pentose phosphates; S-7-P = sedoheptulose 7-phosphate; E-4-P = erythrose 4-phosphate; F-6-P = fructose 6-phosphate; GAP = glyceraldehyde 3-phosphate.

TABLE I

Pentose phosphate formation via the direct oxidative and non-oxidative routes of the pentose phosphate pathway in Krebs ascites cells

| | Oxidation of [$^{14}$C]glucose | | | | Measured pentose phosphate | | | Calculated non-oxidative pentose phosphate |
|---|---|---|---|---|---|---|---|---|
| | [1-$^{14}$C] | [2-$^{14}$C] | [6-$^{14}$C] | C1-C6 | 0 min | 10 min | Net | |
| Control | 0·53 | 0·06 | 0·06 | 0·49 | 0·65 | 2·10 | 1·45 | 0·96 |
| Pyruvate | 0·68 | 0·02 | 0·04 | 0·64 | 0·65 | 5·50 | 4·85 | 4·21 |
| Oxamate | 0·27 | 0·04 | 0·03 | 0·24 | 0·65 | 0·85 | 0·20 | 0·00 |
| Nicotinamide | 0·59 | 0·05 | 0·05 | 0·54 | 0·21 | 1·96 | 1·75 | 1·21 |
| Streptozotocin | 0·38 | 0·08 | 0·07 | 0·31 | 0·83 | 2·54 | 1·71 | 1·40 |

Glucose utilisation by the direct oxidative and non-oxidative reactions was calculated from the $^{14}CO_2$ yields divided by the specific activity of the added glucose. C1 - C6 represents the amount of pentose phosphates produced by the direct oxidative route, and the yield of pentose phosphates from the non-oxidative route was calculated from the difference of the net pentose phosphate synthesised and that produced by the direct oxidative route. Pentose phosphates were assayed directly as described under Methods section. Results are expressed as μmoles/g cells/10 minutes.

Pyruvate caused a marked increase in the accumulation of all the intermediates of the pathway, in particular the pentose phosphates. It seems likely that this could in part be due to an increased flux through the two direct oxidative reactions of the pathway as a result of increased availability of $NADP^+$. The $^{14}C$-glucose data in Table I support the view that there is an increased rate of glucose oxidation via the pentose phosphate pathway. This finding is in accord with the results of Wenner et al. (2).

An approximate estimate of the contribution of the direct oxidative and non-oxidative reactions of the pentose phosphate pathway to the formation of pentose phosphates can be achieved by estimating the rate of formation of $^{14}CO_2$ from [1-$^{14}C$]glucose, the direct oxidative route, and from the total pentose phosphate accumulated during the incubation. The difference between the latter and former values may, as a first approximation, be taken as a measure of the formation of pentose phosphates non-oxidatively. This will depend on how far the accumulated pentose phosphates represent net synthesis. The recycling of pentose phosphates to glucose 6-phosphate and its subsequent reoxidation appears to be minimal since the oxidation of carbon 2 of glucose is almost identical with that of carbon 6, suggesting that there is no alternative route of oxidation of C-2 of glucose over and above the glycolytic pathway and tricarboxylic acid cycle. The incorporation of ribose 5-phosphate into RNA is not yet known in these experiments, but the phosphoribosylpyrophosphate formed would be estimated with the pentose phosphates because of the lability of this compound in $HClO_4$.

From Table I it may be calculated that in the control ascites cells about one third of the pentose phosphates is formed oxidatively and two thirds non-oxidatively. Pyruvate raised the non-oxidative contribution to about 400% compared with about 30% increase from the direct oxidative reactions. It is possible that the increased availability of fructose 6-phosphate could be an important factor in the control of pentose phosphate synthesis by the transketolase-transaldolase reactions.

The converse of the above condition, the effect of oxamate, an inhibitor of lactic dehydrogenase, is also shown in Figure I. Here, there is a general decrease in the concentration of all the metabolites of the pentose phosphate pathway. The fall in 6-PG and pentose phosphates is in keeping with an inhibition of NADPH reoxidation and consequently a decrease in the oxidative reactions of the pathway. This effect of oxamate is not unequivocal, since Elwood has recently shown that oxamate has the additional effect of lowering the hexokinase activity in ascites cells (9). Hexokinase is a very active enzyme in Krebs ascites cells (15 units/g cells), approximately ten times the activity of glucose 6-phosphate and 6-phosphogluconate dehydrogenases, but nevertheless there is a marked decrease in the glucose 6-phosphate content down to 0.13 mM, almost identical with the Km value for glucose 6-phosphate dehydrogenase of these ascites cells (11). Thus substrate as well as enzyme limitation could have played a role in the decreased concentration of metabolites of the pentose phosphate pathway. The oxidation of [1-$^{14}$C]glucose is decreased by about 50%, further evidence for the decreased flux through the direct oxidative reactions of the pathway. Table I shows that the direct oxidative route is sufficient to account for the low rate of accumulation of pentose phosphates and that synthesis by the non-oxidative reactions is apparently completely blocked, probably as a result of the marked fall in fructose 6-phosphate concentration.

The effect of nicotinamide treatment on the metabolite pattern in ascites cells is shown in Figure 2. There is a significant increase in the 6-PG concentration, the pentose phosphates remaining almost unchanged, suggesting an increase in glucose 6-phosphate dehydrogenase activity. This would be in keeping with an increased availability of $NADP^+$, the Km for which is higher for glucose 6-phosphate dehydrogenase than for 6-phosphogluconate dehydrogenase: $2.2 \times 10^{-5}$M and $5 \times 10^{-6}$M respectively (11). Preliminary experiments have shown a 36% increase in the total NADP content of these ascites cells after treatment with nicotinamide. The pattern of oxidation of

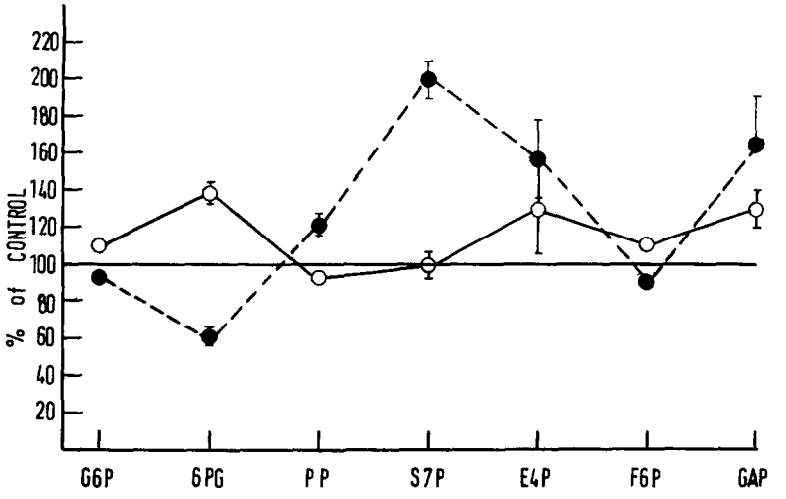


Figure 2. Effect of nicotinamide and streptozotocin on levels of intermediates of the pentose phosphate pathway in Krebs ascites cells.

Open circles represent nicotinamide effect and closed circles streptozotocin effect. Each point represents the mean of at least six observations.

specifically-labelled glucose did not yield evidence for increased oxidation of glucose by way of the direct oxidative or the non-oxidative routes.

Streptozotocin treatment caused a marked lowering of the 6-PG content of the ascites cells (Figure 2) which may possibly be ascribed to an effect on glucose 6-phosphate dehydrogenase of a limitation of $NADP^+$ availability. There was approximately a 40% decrease in the rate of oxidation of carbon 1 of glucose (Table I). The rise in pentose phosphates, sedoheptulose 7-phosphate and erythrose 4-phosphate suggests an increased rate of their synthesis by the non-oxidative reactions of the pathway.

An important question is whether the pentose phosphate pathway operates as a cycle or as direct oxidative and non-oxidative sequences functioning independently, both routes being important in the synthesis of pentose phosphates. From the above experiments, it may be inferred that the major route of pentose phosphate synthesis is the non-oxidative route, that is by

way of transketolase and transaldolase reacting in reverse to the direction of the direct oxidative route. These findings are in fair agreement with the observations of Hiatt (12).

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