Table 1. Growth Inhibition of *E. coli* by Thymine Xyloside and its Reversal by Glutathione

| Thymine xyloside concentration (μgm./ml.) | Glutathione concentration necessary to reverse inhibition (μgm./ml.) | Ratio |
|---|---|---|
| 100 | 12·3 | 8/1 |
| 33 | 3·2 | 10/1 |
| 10 | 0·8 | 12/1 |

In a search for a possible reversing agent, glutathione was tried. This proved extremely effective in reversing the inhibition. About 0·1 mole of glutathione reversed 1 mole of thymine xyloside and this reversal was competitive (Table 1).

Reversal studies with purine and pyrimidine bases and nucleosides revealed no significant effects of these compounds. Fair reversal was obtained with technical casamino-acids ('Difco'). A survey of amino-acids revealed no single one which was as active as the casamino-acids. No agent was found which was as effective as glutathione in reversing the inhibition.

This competitive relationship between thymine xyloside and glutathione is quite interesting, and there is no apparent simple biochemical explanation for it. Beltz and Visser[1] have commented that 5-substituted deoxyribosyl-pyrimidines are the only substituted nucleosides which inhibit the growth of micro-organisms not requiring purines and pyrimidines for growth. Since thymine xyloside is a 5-substituted nucleoside with an unnatural sugar, it may be inhibiting in an analogous manner. It might be possible to use thymine xyloside to induce glutathione deficiencies so that glutathione metabolism and function can be more readily studied.

The thymine xyloside used in these studies was prepared by the method of Fox *et al.*[2] and was kindly supplied by Dr. J. H. Hunter of these Laboratories. Dr. Seymour Cohen has given helpful advice and encouragement.

Thomas D. Brock*

Research Laboratories,
The Upjohn Co.,
Kalamazoo, Michigan.

* Present address: Department of Biology, Western Reserve University, Cleveland 6, Ohio.

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## Aerobic Inhibition of Glycolysis

During investigations on the synthesis of sucrose from glucose-1-phosphate and fructose by pea seed extracts it was observed that a glycolytic system was operating in the enzymic digests[1,2]. The pea extract had been partially purified by precipitation with ammonium sulphate (80 per cent saturation) and dialysis against phosphate buffer at *p*H 7·0. It was found that there were differences in glycolytic activity when the enzymic digests were incubated under aerobic and anaerobic conditions.

When digests containing glucose-1-phosphate and fructose were incubated in air there was a small increase in inorganic phosphate after 40 hr. (Table 1). In nitrogen the inorganic phosphate decreased. Visual examination of the phosphoric compounds of the digest samples on paper chromatograms developed in *n*-propyl alcohol/ammonia/water (60 : 30 : 10, v/v) and *tert*-amyl alcohol/water/formic acid (90 : 90 : 30, v/v)[3] confirmed this decrease in inorganic phosphate and showed that extra phosphoric esters

Table 1. Effect of Anaerobic Conditions on Inorganic Phosphate Content

| Gas phase | Inorganic phosphate (μmoles/ml. digest) | |
|---|---|---|
| | 0 hr. | 40 hr. |
| Air | 9·5 | 11·5 |
| Nitrogen | 9·5 | 2·2 |

Digest: glucose-1-phosphate, 0·0144 *M*; fructose, 0·0244 *M*; $MgCl_2$, 0·01 *M*; yeast nucleotide fraction, 0·5 ml.; partially purified pea extract, 1 ml.; total volume, 1·8 ml. *p*H 7·0, 25° C.

Table 2. Effect of Anaerobic Conditions on Fructose Loss

| Gas phase | Fructose (μmoles/ml. digest) | |
|---|---|---|
| | 0 hr. | 20 hr. |
| Air | 24·4 | 10·1 |
| Evacuated | 24·4 | 0 |

Digest: as in Table 1.

accumulated in nitrogen. This accumulation appeared to be largely due to increases in fructose-1,6-diphosphate, hexose monophosphates and a substance running in a position similar to adenosine triphosphate. Incubation of the enzymic digests *in vacuo* gave similar results to those obtained in nitrogen.

Anaerobic conditions also accelerated the decrease in fructose in the enzymic digests (Table 2). Fructose had disappeared after 20 hr. under anaerobic conditions, whereas in air a considerable quantity of this sugar remained.

When the gas exchange of the enzymic digests was examined, it was found that in air there was a barely significant oxygen uptake suggesting that oxidative processes were not proceeding to an appreciable extent. There was a considerable carbon dioxide output in air, and this was increased markedly in an atmosphere of nitrogen.

The effect of oxygen on the enzymic digests may be analogous to the Pasteur effect, for which a number of explanations have been proposed[4-6]. If so, the results obtained in the present investigation may support the hypothesis of Lipmann[7] that oxygen inhibits glycolysis by inactivating one or more of the glycolytic enzymes. In the simple system investigated here (which is not complicated by the existence of respiratory processes) glycolysis is the only source of adenosine triphosphate or energy-rich phosphate bond. It seems probable that the rate of hexose utilization is decreased under aerobic conditions because of a decreased production of adenosine triphosphate and this may in part be due to a partial inactivation of one of the glycolytic enzymes.

J. F. Turner*

(Broodbank Fellow of the University of Cambridge)
Botany School,
Cambridge.

L. W. Mapson

Low Temperature Station for
Research in Biochemistry and Biophysics,
University of Cambridge and Department
of Scientific and Industrial Research.

* Present address: Division of Food Preservation and Transport, C.S.I.R.O., Botany School, University of Sydney.

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