Accumulation of nucleic acid metabolites in *Escherichia coli* exposed to the action of 6-azauracil

As we have shown some time ago, 6-azauracil (3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine) inhibits the growth of bacteria[1,2]. The antibacterial action of 6-azauracil has also been established independently by HANDSCHUMACHER AND WELCH[3]. Later we were able to show[4] that 6-azauracil is converted to 6-azauracil riboside by *E. coli*; the extent of this metabolic reaction is such that it could be utilized preparatively for the synthesis of the riboside[5]. 6-Azauracil riboside has also been detected in cells of *Streptococcus faecalis* grown in the presence of slightly inhibitory levels of 6-azauracil[6]. Since 6-azauracil has also been found to possess carcinostatic activity[7-9], we undertook the preparation of larger amounts of 6-azauracil riboside by the microbiological method mentioned above[10] for testing against experimental tumours. Although 6-azauracil riboside is less potent an inhibitor of *E. coli* than 6-azauracil, the carcinostatic action of the riboside greatly exceeds that of free 6-azauracil[11]. Inhibition of the growth of Sarcoma 180 in tissue culture by 6-azauracil riboside had been described by SCHINDLER AND WELCH[12].

In the course of our work on the fermentative synthesis of 6-azauracil riboside we have isolated a number of products from the culture medium and the bacterial cells whose occurrence there sheds some light on 6-azauracil inhibition.

E. coli B was cultivated on a synthetic medium containing inorganic salts and glucose[5], with aeration and stirring. 6-Azauracil (10^{-3} *M*) was added to the culture at the beginning of the logarithmic phase. After cultivation for 4.5 h in the presence of the inhibitor, the bacterial cells were separated (Sharples centrifuge) and extracted with 5% trichloracetic acid (TCA) in the cold. The extract was freed from trichloracetic acid by ether extraction and the nucleic acid metabolites isolated by means of active charcoal. Similarly, active charcoal was used to obtain the nucleic acid metabolites from the filtered culture medium. The metabolites were identified by paper chromatography in the butanol–water and *sec.*-butanol–HCl–water (43:11:9) systems using authentic standards. The identification was confirmed by a comparison of the ultraviolet spectra of the chromatographically isolated metabolites with those of reference samples.

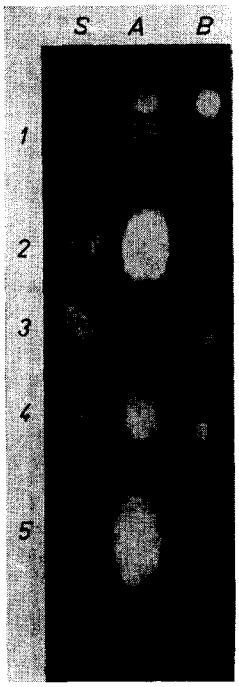

Fig. 1. Chromatograms of the nucleic acid metabolites isolated from the medium (A) and trichloracetic acid extract of the cells (B) after cultivation of *E. coli* in the presence of 6-azauracil. Whatman No. 1 paper, developed by butanol saturated with water, photographed by u.v. light. Standards (*S*): 1, orotic acid; 2, 6-azauracil riboside; 3, hypoxanthine; 4, uracil; 5, 6-azauracil. The orotic acid present in the medium crystallised after elution from the active charcoal and is therefore missing on the chromatogram. The nucleotides present in the TCA extract remain at the start.

It was found that during the cultivation of *E. coli* in the presence of 6-azauracil considerable amounts of orotic acid accumulate in the medium in addition to 6-azauracil riboside. Further, free uracil and hypoxanthine were found to be present (Fig. 1A). The cell extract contained free uracil and hypoxanthine in addition to small amounts of orotic acid and 6-azauracil riboside; free azauracil was present only in traces (Fig. 1B).

The amount of orotic acid and 6-azauracil riboside accumulated in the medium is considerable—so much so, in fact, that *E. coli* growing in the presence of 6-azauracil may be advantageously used for the biosynthesis of these compounds isotopically labelled with ^{14}C or ^{15}N.

As we have shown earlier, 6-azauracil is a competitive antagonist of uracil, cytosine, and their ribosides in the bacterial cell. We expressed the view that the 6-azauracil is converted to riboside metabolites which interfere with nucleic acid synthesis[2]. The accumulation of intermediary ribonucleic acid metabolites reported in this paper, together with the formation of the riboside of the inhibitor, are in line with this assumption.

The occurrence of free hypoxanthine is evidence for the view that this compound is a precursor of the nucleic acid adenine and guanine in microorganisms (where biotin deficiency also causes the accumulation of hypoxanthine[13]) as well as in animal tissues[14].

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these and related experiments will be given in due course in the Collection of Czechoslovak Chemical Communications.

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On the importance of α-glycerophosphate dehydrogenase in glycolysing insect muscle*

The DPN-linked α-glycerophosphate dehydrogenase catalyzes the reduction of dihydroxyacetone phosphate according to the reaction:

$$\text{Dihydroxyacetone phosphate} + \text{DPNH} \rightleftarrows \alpha\text{-glycerophosphate} + \text{DPN}^+$$

This reaction is of little significance in vertebrate-muscle glycolysis because it is the reduction of pyruvate which is linked with the oxidation of glyceraldehyde-3-phosphate. However, it is known that glycolysis in insect muscle lacks the proper stoichiometry[1,2], which may be due to the impaired activity of lactic dehydrogenase[3].

The activities of lactic and α-glycerophosphate dehydrogenases in both coxal and thoracic muscles of the American cockroach, *Periplaneta americana*, are seen in Table I.

The results clearly show that the lactic dehydrogenase level in both types of muscle is unusually low. On the other hand, that of α-glycerophosphate dehydrogenase is extraordinarily high. Consequently, most of the DPN is probably regenerated by the reduction of dihydroxyacetone phosphate.

In order to check the physiological significance of these activities, the formation of lactate, pyruvate and α-glycerophosphate from hexose diphosphate was studied under anaerobic conditions. Typical results presented in Table II indicate the stoichiometry of hexose diphosphate conversion.

For every mole of HDP utilized, one mole of α-glycerophosphate and pyruvate was formed. The slower rate of accumulation of pyruvate suggests that the reactions producing pyruvate *via* the Embden-Meyerhof scheme appear to be operating but one or more may be rate-limiting.

The conversion of HDP required DPN which could not be replaced by TPN. ADP alone and ADP plus inorganic phosphate had a slightly stimulatory effect on the metabolism of HDP.

In summary, therefore, the reductive step represented by lactic dehydrogenase appears to be of negligible importance in glycolysis of coxal muscles of the roach. Instead, the α-glycerophosphate dehydrogenase system assumes the major role in regenerating DPN from DPNH. The predominant end-products of roach-muscle glycolysis appear to be α-glycerophosphate and pyruvate. Consequently we have here a normal situation similar to Neuberg's so-called second form of fermentation which may be also induced in muscle with the aid of "steering substances"[8].

* The following abbreviations are used: DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; ADP, adenosine diphosphate; HDP, hexose diphosphate; TPN, triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.