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THE DEMONSTRATION OF THE SITE OF ACTION OF THE ANTIMETABOLITE DRUG 6-AZAURIDINE BY THE USE OF LEUCOCYTE CULTURES

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The ability of the lymphocyte cultured *in vitro* to transform and synthesize deoxyribonucleic has been utilized to locate the site of action of a new antimetabolite 6-azauridine which acts by inhibiting nucleic acid synthesis. The exact site can be demonstrated by incorporating into the cultures nucleotides occurring distal and proximal to the presumed site of action of 6-azauridine.

6-Azauridine, a pyrimidine analogue, has recently been investigated at several centres as an experimental drug for the treatment of human leukaemias. It is unique among antimetabolites in its ability to kill certain types of neoplastic cell in man and to have only slightly harmful effects on most normal cells *in vivo* (Welch, Handschumacher, Finch, Jaffe, Cardoso & Calabresi, 1960).

In order to investigate the site of action of the drug we have utilized the techniques of lymphocyte culture in the presence of phytohaemagglutinin, a bean extract causing lymphocytes to undergo transformation to large primitive cells (Nowell, 1960; Elves & Wilkinson, 1963). During this transformation the lymphocytes carry out synthesis of deoxyribonucleic acid, as is revealed by autoradiography (Cooper, Barkham & Hale, 1961) and by metabolic tracer studies (MacKinney, Stohlmann & Brecher, 1962). As 6-azauridine acts in the deoxyribonucleic acid synthetic pathway (see Fig. 1) it presumably inhibits lymphocyte transformation, and by using

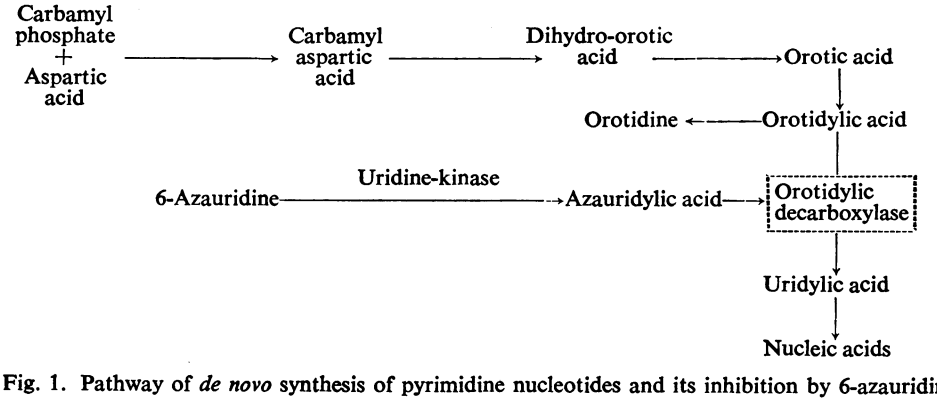

Fig. 1. Pathway of *de novo* synthesis of pyrimidine nucleotides and its inhibition by 6-azauridine.

it in combination with deoxyribonucleic acid precursors in these cultures it should be possible to demonstrate the site at which 6-azauridine acts. To this end we have used orotic acid, orotidine and uridylic acid.

METHODS

Leucocyte cultures were prepared from peripheral blood of normal healthy donors by the method of Hungerford, Donnelly, Nowell & Beck (1959), using phytohaemagglutinin (P-form, Difco) at a concentration of 0.03 ml./culture. Dextran was used to aid sedimentation of the red cells and to obtain a leucocyte-rich plasma. The drugs tested, in concentrations shown in Table 1, were added to the culture medium and after 72 hr of incubation at 37° C the cells

TABLE 1

"BLAST" TRANSFORMATION AFTER 72 HR OF INCUBATION AT 37° C

Each culture contained phytohaemagglutinin (0.03 ml./culture)

Drugs present	Dose (μg/ml.)	Blast transformation (%)	Inhibition of blast transformation (%)
None (control)	—	26–30	0
Azauridine	100	2	93
Azauridine	50	4	87
Orotic acid	100	27	0
Orotidine	100	30	0
Uridylic acid	100	27	0
6-Azauridine+orotic acid	50+ 50	3	89
6-Azauridine+orotic acid	50+100	3	89
6-Azauridine+orotic acid	50+200	5	82
6-Azauridine+orotidine	50+ 50	5	82
6-Azauridine+orotidine	50+100	4	87
6-Azauridine+uridylic acid	50+ 50	26	4
5-Azauridine+uridylic acid	50+100	30	0

were separated and spread upon slides and stained with Jenner-Giemsa stain. Differential counts of 200 lymphoid cells were then made and the percentage of transformed lymphocytes was determined. The degree of inhibition was calculated by comparing the percentage of transformed cells in cultures with added drug with the percentage found in controls.

RESULTS

The "blast" transformation and the degree of inhibition calculated from it in each of the various cultures are shown in Table 1. The normal deoxyribonucleic acid precursors, orotic acid, orotidine and uridylic acid, had no effect on the degree of lymphocyte transformation. 6-Azauridine, on the other hand, caused a profound depression in the degree of transformation which could only be prevented if uridylic acid was included in the medium of cultures treated with 6-azauridine. The other two precursors were not able to block the effect of the antimetabolite.

DISCUSSION

Two areas of pyrimidine metabolism have been postulated as the primary sites of action of 6-azauridine. Elion, Bieber, Nathan & Hitchings (1958) have suggested that azauracil, or its ribonucleoside, acts as an antagonist of uracil anabolism at

some stage between uracil and uridine triphosphate. In support of this hypothesis Skoda & Sorm (1959) have shown that 6-azauridine competes to some extent with uridine as a substrate for the enzyme uridinekinase of *Escherichia coli B.* The other postulated site of action of 6-azauridine presumes that it acts, after phosphorylation, as a competitive inhibitor of the enzyme orotidylic decarboxylase, thus creating a profound impairment of the *de novo* synthesis of nucleic acids (Handschumacher, 1960). This site of action of 6-azauridine is shown in Fig. 1.

The technique used in these studies relies upon the morphological changes in the small lymphocyte under conditions in which it is actively synthesizing deoxyribonucleic acid. Our experiments show quite clearly the potent antimetabolic effect of 6-azauridine. The finding that this effect may be overcome by providing the cells with uridylic acid demonstrates that the site of action of 6-azauridine is proximal to the occurrence of this substance in the chain of pyrimidine biosynthesis. The inability of the other two precursors, orotic acid and orotidine, to prevent the damage caused by the 6-azauridine in these cultures indicates that the drug acts distally to the occurrence of these nucleotides in the chain. From these results it is highly likely the 6-azauridine acts on the stage at which orotidylic acid is converted to uridylic acid, which is catalysed by the enzyme orotidylic decarboxylase.

The above results do not, however, contradict the findings of Elion *et al.* (1958) that both uracil and uridine could prevent the toxic effects of azauracil. In fact any pyrimidine nucleotide distal to the site of action of 6-azauridine, such as uracil or uridine, could act as a potential reversing agent by circumventing the block imposed by 6-azauridine.

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