Studies concerning inhibition of the synthesis of deoxycytidine by phosphorylated derivatives of thymidine

As described for other mammalian cells[1,2] the murine neoplastic cell lines, L5178Y and P815Y, require thymidine for reproduction in culture if synthesis *de novo* of this compound is inhibited with either 5-fluoro-2′-deoxyuridine or amethopterin. Under these conditions, and with successive increases in the concentration of thymidine in the medium, cellular reproduction is increased progressively until the extent of growth of the cells equals or even surpasses that of the controls; however, further increments in the amounts of added thymidine progressively inhibit growth (Fig. 1). The inhibitory phases of this response can be demonstrated equally well in the absence of either antimetabolite.

The recent observation that the triphosphate of either thymidine, deoxyadenosine or deoxyguanosine inhibits the conversion of the ribonucleotide, cytidine 5′-phosphate to the corresponding 2′-deoxy compound[4] suggested that the inhibition of growth

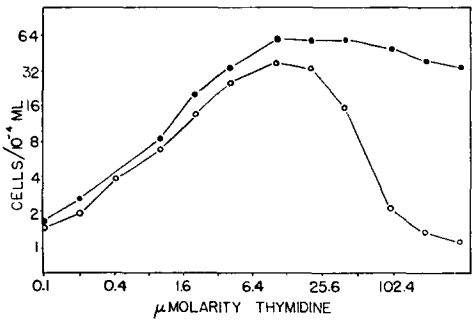

Fig. 1. The support and subsequent inhibition of growth by thymidine in the presence of $4 \cdot 10^{-8}$ *M* FUDR (40 times the 50 % inhibitory level). Culture techniques and media are similar to those described by FISCHER[3]. Tubes were inoculated with 10^4 cells/ml medium and the number of cells was determined, using a Coulter counter, after incubation for 72 h. 2′-Deoxycytidine, to give a final concentration of $4 \cdot 10^{-5}$ *M*, was added to the tubes represented by closed circles. None was added to those tubes represented by open circles.

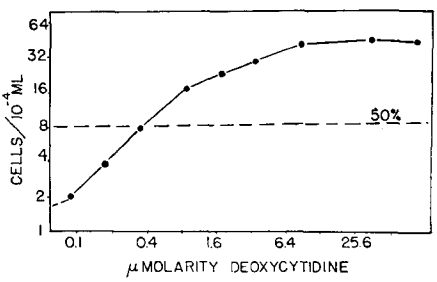

Fig. 2. Reversal by 2′-deoxycytidine of thymidine-induced inhibition of reproduction of L5178Y cells. Each tube contained $1.8 \cdot 10^{-3}$ *M* thymidine plus 2′-deoxycytidine as noted; 50 % growth was supported by $6 \cdot 10^{-7}$ *M* 2′-deoxycytidine. Similar results were obtained with P815Y cells and 50 % growth was supported by $6 \cdot 10^{-6}$ *M* 2′-deoxycytidine.

by thymidine might be accounted for in a similar manner, and a study of the effect of 2′-deoxyribo nucleosides on the toxicity of thymidine was indicated. 2′-Deoxycytidine alone at low levels was found to prevent thymidine inhibition (Fig. 1). However, 2′-deoxyuridine, 2′-deoxyadenosine or 2′-deoxyguanosine, either singly or in combinations at $5 \cdot 10^{-4}$ *M* and at $5 \cdot 10^{-5}$ *M* were inactive. In the presence of an otherwise inhibitory high concentration of thymidine ($1.8 \cdot 10^{-3}$ *M*), half-maximal growth is obtained by the addition of a low concentration of 2′-deoxycytidine of the same order as that required with thymidine ($1 \cdot 10^{-6}$ *M*) for growth in the presence of the above-mentioned antimetabolites (see Fig. 2). Cytidine, even at a concentration of $4 \cdot 10^{-4}$ *M*, has little or no effect on the inhibition of growth by thymidine.

Presumably thymidine inhibits growth only after its conversion to the 5′-

Abbreviation: FUDR, 5-fluoro-2′-deoxyuridine.

TABLE I

RATE OF PHOSPHORYLATION OF THYMIDINE BY EXTRACTS OF FUDR-SENSITIVE AND FUDR-RESISTANT MAST CELLS

0.25 ml of supernatant (100,000 × *g* for 1 h) was added to 1.0 ml of a mixture compounded such that these final concentrations were obtained: $8 \cdot 10^{-5}$ *M* [^{3}H]thymidine (9 μC/μmole), $4.2 \cdot 10^{-3}$ *M* ATP, $6 \cdot 10^{-3}$ *M* 3-phosphoglycerate, $4.8 \cdot 10^{-3}$ *M* Mg^{++} and $1.25 \cdot 10^{-1}$ *M* tris(hydroxymethyl)-aminomethane, pH 8.0. Incubations were carried out at 36° and terminated by chilling and addition of 0.1 ml 70% $HClO_4$. $KClO_4$ was precipitated prior to the separation of phosphorylated compounds from thymidine using Dowex 1 × 10 formate[6]. Activity is expressed as moles product/min/mg protein.

Sensitive (P815Y)	*Resistant (P815Y/FUDR)*
$1 \cdot 10^{-10}$	$3 \cdot 10^{-12}$

phosphate, since a strain of P815Y selected for high resistance to FUDR[9], which is apparently deficient in thymidine kinase activity (Table I), is not inhibited by thymidine[5]. The results suggest that in sensitive cells a phosphorylated derivative of thymidine inhibits the conversion of cytidine 5′-phosphate to 2′-deoxycytidine 5′-phosphate, thereby limiting DNA synthesis and cell division.

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Biological oxidation of N,N-dialkyl carbamates

N-Alkyl and N,N-dialkyl carbamates are widely used as insecticides [1,2] and medicinals for the relief of myasthenia gravis and other disorders[3]. The ultimate mode of action appears, in both cases, to be the competitive inhibition of cholinesterase. Cholinesterase[4] and plasma albumin[2,5] have been implicated in the biological degradation of N-alkyl carbamates. N,N-dimethyl carbamoyl fluoride is hydrolyzed by the plasma A-esterase and certain other esterases in the rabbit[6]. The N,N-dialkyl carba-

Abbreviations: DpNC, N,N-dimethyl *p*-nitrophenyl carbamate; TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; SKF 525-A, β-diethylaminoethyl diphenylpropylacetate. The chemical designations for insecticides and synergists are given in papers cited[1,2,10,11].