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SHORT COMMUNICATIONS

The effect of 2-thiouracil on the division of phytohaemagglutinin-stimulated human lymphocytes *in vitro*

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2-THIOURACIL (2TU) is a well known antimetabolite with inhibiting effects on the growth of a number of organisms (Ber,[2] Standskov and Wyss,[9]; Moore,[8]; Klein and Klein,[7]). Hamers[4] showed that 2TU replaced about 20 per cent of the uracil in the RNA of *Bacillus megatherium* after 3 hr exposure and that there was also partial inhibition of growth. It would appear that 2TU has two possible modes of action in the biochemistry of the cell (a) interference with the UDP/UTP system and (b) interference with enzyme synthesis by alteration of the RNA coding sequence. The reported gross effects may well be due to a combination of both modes of action as they are not mutually exclusive. The effect of 2TU on human cells is of particular importance because of the therapeutic use of this compound and its derivatives in the treatment of hyperthyroidism.

MATERIALS AND METHODS

Blood samples were obtained from volunteers with no history of any thyroid disorder or treatment with 2TU or its derivatives.

The technique used for chromosome cultures was as follows. About 20 ml of venous blood was mixed with 2 ml of anticoagulant (heparin in dextran) in a sterile container. The red cells were allowed to settle and the supernatant plasma and leucocytes were drawn off. The cultures were made up to 10 ml with TC 199 culture medium (Glaxo) to give a final concentration of about 10^6 cells per ml. Two drops of phytohaemagglutinin P (PHA, Difco) were added to each culture. The cultures were incubated for 72 hr at 37°. At this stage 0·2 ml of "Colcemid" (demecolcine) at a concentration of 1 mg/100 ml of TC 199 was added and the cultures were incubated for a further 1½–2 hr. The cultures were then spun down at about 2000 rev/min for 10 min and the supernatant discarded. The cells were resuspended in about 5 ml of hypotonic saline, 0·25% (w/v), for 15 min at 37°, fixed with acetic alcohol (1 part glacial acetic acid to 3 parts ethanol), resuspended in sufficient 45% acetic acid to give an opalescent suspension, spread on to cold slides and air-dried. The preparations were all stained with 10% Giemsa at pH 6·4.

The experimental cultures were exposed to 2TU either (a) throughout the 72 hr or (b) by using 2TU in place of the "colcemid" at 72 hr. In a third series of experiments the cultures were first treated as in (b), then after 90 min exposure to 2TU at 37° about 90 per cent of the culture medium was withdrawn and replaced with fresh TC 199 and two drops of PHA added. These cultures were then incubated for a further 72 hr at 37° and then treated with "colcemid", fixed, spread and stained in the usual manner. This is basically the technique used by Herreros, Guerro and Romo[6] to obtain polyploid cells using "colcemid" in human peripheral blood cultures.

RESULTS AND DISCUSSION

The Mitotic Index here defined as the number of mitoses per 1000 cells was determined for each concentration used and the results expressed as percentages of the values obtained for the controls. In Table 1 each treatment column represents the averaged results of three experiments.

The effect of 2TU in the first series of experiments was to cause a depression of the mitotic index by about 50 per cent at quite low concentrations which are similar to those to be expected in patients on treatment with 2TU or one of its derivatives. The absence of a concentration effect may indicate the existence of a threshold which has been reached at these concentrations.

In the second series, however, a definite concentration effect was observed. The depression of the mitotic index was almost doubled when the concentration of 2TU was increased by a factor of 100. The third series showed no concentration effect but the depression of the mitotic index was much greater, averaging 83 per cent.

TABLE 1. THE EFFECT OF 2TU ON CULTURED HUMAN LYMPHOCYTES
Each treatment column represents the average of three experiments.

Concentration of	With 2TU in cultures*		With 2TU used in place of "colcemid"†		2TU Treatment at 72 hr. Cultured a further 72 hr.†	
2-Thiouracil	Mitoses /1000 cells	Expressed as per cent of control	Mitoses /1000 cells	Expressed as percent of control	Mitoses /1000 cells	Expressed as percent of control
10^{-3}M	6·6	52·4	5	27·8	3	16·7
10^{-4}M	4·6	36·5	8	44·4	4	22·2
10^{-5}M	7·2	57·1	9	50·0	2	11·1
Control	12·6	100	18	100	18	100

* Significant at 5 per cent level.
† Significant at 0·1 per cent level.

The side effects of the therapeutic use of 2TU and its derivatives are well established (Goodman and Gilman[3]; Beeson and McDermott[1]; Tumulty and Cluff in Harrison[5]; Williams[10]). Estimates of reactions vary between 0·5 per cent and 13·8 per cent with agranulocytosis being the most common. The results reported here would explain the agranulocytosis, the basic cause being a reduction in the number of circulating leucocytes as a result of mitotic depression. It may also account for some of the other reactions reported. This reduction in mitosis may be assumed to occur in all patients treated with 2TU or one of its derivatives although in most cases this appears to be without clinical manifestation.

It must be emphasised that the effect of 2TU presented here is on PHA-stimulated lymphocytes there being no direct evidence that it has the same effect on spontaneous growth.

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