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Discussion of Dr. Farber's paper

I SHOULD like to contrast Dr. Farber's experiments, on the effect of inhibition of DNA synthesis in intestinal cells, with our experiments on the consequences of inhibiting DNA synthesis in various cell types growing in tissue culture. We can summarize our findings on the effects of alkylating agents on dividing cells as follows.[1, 2] Cells treated with mustard gas during the G2 phase of the cell cycle pass through mitosis without delay. The daughter cells enter the next DNA synthetic period quite normally but then show an inhibition in the rate of DNA synthesis. Following this inhibition there is a prolongation of the S period with a subsequent delay in mitosis. Both the inhibition in the rate of DNA synthesis and the extent of delay in cell division are dose-dependent. Cells treated during the G1 phase of the cell cycle also enter the S phase quite normally but show a dose-dependent inhibition in the rate of DNA synthesis and a subsequent dose-dependent delay in mitosis. Cells treated during S show an immediate depression in the rate of DNA synthesis and a delay in cell division. The monofunctional agent, half-mustard gas elicits a similar response in cells[3] as shown in Fig. 1, which shows the effect no the rate of DNA synthesis in cells treated early in G1. Following the depression in rate of DNA synthesis and the resultant delay in division the cells would seem to have recovered from the consequences of alkylation, since there is no further delay in division. The time between the second and third DNA synthetic periods following treatment is the same as in the control. The level of monofunctional alkylation required to produce these effect is, however, approximately ten times the level of reaction of the

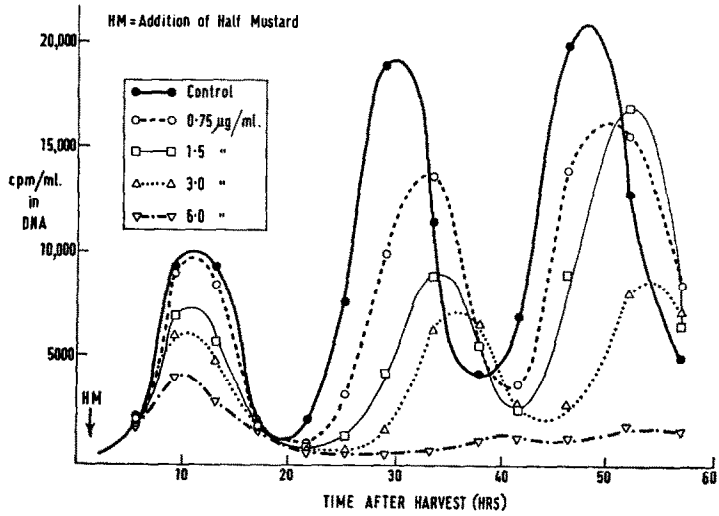

FIG. 1. Shows the effect of half sulphur mustard (2-chloroethyl-2′-hydroxyethyl sulphide) on DNA synthesis in a synchronized population of HeLa cells. Mitotic cells were harvested from a confluent monolayer culture of HeLa cells by the method of Robbins and Marcus[4] and placed in a stirred suspension culture at a cell concentration of approximately 5×10^4/ml. Replicate cultures were treated with half mustard at concentrations of 0·75, 1·5, 3·0 and 6·0 μg/ml in ether solution, 0·2 μl/ml, 1 hr after harvesting. These concentrations were equivalent to approximately 60%, 10%, 0·1% and negligible survival, respectively. Duplicate 1 ml aliquots of cells were withdrawn after various times and incubated with [^{3}H]thymidine, 0·5 μc/ml, 17·7 c/m-mole, for 20 min. The cell suspension was then rapidly diluted with cold phosphate buffered saline (P.B.S.) and filtered onto a millipore filter, which was then washed with cold P.B.S. and cold 5% trichloroacetic acid. The millipore filter was dissolved in scintillation fluid and the radioactivity incorporated into cellular DNA counted in a Tricarb liquid scintillation counter. The average of the counts from duplicate samples was plotted against time after harvest.

corresponding difunctional agent, mustard gas. It is clear that these effects are the consequence of the alkylation of DNA *per se* and not due to reaction with other cell constituents. At the level of alkylation with mustard gas which produces an inhibition in rate of DNA synthesis and subsequent inhibition of cell division the level of alkylation of protein is such that only 1 protein molecule in approximately 2000 molecules will be alkylated assuming a molecular weight of 100,000. Clearly, unless one postulates the selective alkylation of a particular protein enzyme, and one concerned in DNA replication, these effects on DNA synthesis cannot be the result of inactivation of protein enzymes. Moreover, thymidine kinase and DNA polymerase, as well as total protein and RNA synthesis, have been shown not to be affected unless cells are treated with concentrations of mustard gas very much greater than those required to affect DNA synthesis and cell division. The inhibition of DNA synthesis and mitotic delay leads to the formation of giant cells owing to the continued synthesis of protein and RNA and it is this "out of phase" synthesis which may be regarded as the eventual cause of death in these cells, although the process may take many hours. The rapid lethal effect of HN2 on cells, reported by Dr. Farber, must therefore be the consequence of a quite different mode of action. One cannot readily envisage a mechanism by which protein synthesis inhibitors can reverse the lethal effect of HN2 if it is a consequence of the inhibition of DNA synthesis. Possibly HN2 induces a lytic action on cells and it is this which is reversed by a protein synthesis inhibitor.

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