BIOLOGICAL ACTION OF 'MUSTARD GAS' COMPOUNDS

Mutagenic Activity of beta-Chloroalkyl Amines and Sulphides

IN attacking the problem of the mechanism of biological action of mustard gases, particularly their mutagenic and anti-carcinogenic effects, we have determined the activity of a series of beta-chloroethyl amines and sulphides containing only one reactive grouping. A study of such types of molecules (I and II) is of particular interest in exploring the relation of chemical structure to biological activity. Some monochloroethyl sulphides show the vesicant property of mustard gases. On the other hand, there are indications that mutagenic and anti-carcinogenic activities may be confined to molecules containing two or more reactive groupings[1-3].

$RSCH_2CH_2Cl$ (I) $\qquad$ $R_2NCH_2CH_2Cl$ (II)

In the present study, the mutagenic activity[4] of the compounds has been tested using the technique described by Dickey *et al.*[5]. In this method nutritionally deficient mutant strains of *Neurospora crassa* are used. The activity of a substance in causing reversions to the non-deficient state is measured. Results of typical experiments are given in the accompanying table. The 'induced mutation rate'[5] is the number of mutants produced divided by the total number of spores treated. In all cases, the tests were carried out in 10 per cent aqueous acetone, 0·25 *M* in sodium chloride, and 0·1 *M* in borate (*p*H 8). The treatments were made at 25° for 30 min., using three to four million spores per millilitre.

MUTAGENIC ACTIVITY OF MUSTARD-TYPE COMPOUNDS

Compound	Conc. (*M*)	Induced mutation-rate ($\times 10^7$)	
		Adenine-less mutant (70007-38701)	Inositol-less mutant (70007-37401)
None	—	(0·3*)	(0·0)
$ClCH_2CH_2SCH_2CH_3Cl$	0·0002	4	—
	0·0001	3	1
n-$C_4H_9SCH_2CH_2Cl$	0·0002	64	1
	0·0001	11	—
$C_6H_5CH_2SCH_2CH_2Cl$	0·0002	12	—
$C_6H_5SCH_2CH_2Cl$	0·0002	2	—
$N(CH_2CH_2Cl)_3$	0·0002	2	—
$CH_3N(CH_2CH_2Cl)_2$	0·001	4	2
	0·0005	2	2
$(CH_2CH_2)_2NCH_2$ CH_2Cl	0·006	13	0·5

* Average of all control experiments. In no case was the spontaneous mutation-rate greater than $1·0 \times 10^{-7}$.

It will be noted that the response of the two mutant strains is distinctly different. For the adenine-less, colonial double mutant (70007–38701), the monofunctional sulphur mustards were even more active than mustard gas itself. This was true also of the monofunctional nitrogen mustards when the compounds were compared at the concentration giving maximum activity. Toward the inositol-less, colonial double mutant (70007–37401), on the other hand, both the monofunctional and polyfunctional nitrogen and sulphur mustards showed slight activity.

The finding that these monofunctional mustard-type molecules have pronounced mutagenic activity is of importance to the study of the mechanism of production of mutations. It provides a clue to the relation of structure to mutagenic activity, and also a simpler molecular type for chemical studies[6].

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Production of Mutations by Monochloro-'Mustards'

IN 1942 it was noticed[1] that 'mustards' with one reactive chlorine atom are less toxic than those with two, to a degree which cannot be accounted for by the difference in chlorine content. More recently, workers at the Chester Beatty Institute in London, in the course of an extensive comparative study of nitrogen mustards and related compounds, have made the same observations for the tumour-inhibiting and cytotoxic activities of these substances[2-4]. They conclude that the presence of 2-chloroethyl chains is a necessary condition for biological activity in this class of chemicals, and they suggest that chromosome breakage and mutation arise through cross-linkage between protein fibres of the chromosome. It seemed desirable to test this hypothesis by mutation experiments with some monochloro-'mustards'.

The experiments were carried out on *Drosophila melanogaster*, using a standard technique for the scoring of induced sex-linked lethals. Two substances were used: (1) the first hydrolysis product of mustard gas, $ClCH_2.CH_2.S.CH_2.CH_2OH$, referred to as 'semi-*H*', and (2) 'butyl-*H*', $CH_3.CH_2.CH_2.CH_2.S.CH_2.CH_2Cl$. Both these substances had already been used in experiments by Kinsey and Grant[5] on yeast cells, and had been found to resemble mustard gas in the production of an irreversible growth inhibition, not paralleled by the effect on carbohydrate metabolism. Moreover, in experiments by Stevens[6], butyl-*H* had produced reverse mutations in *Neurospora*. We have to thank Mr. J. L. Everett, of the Chester Beatty Institute, for preparing the semi-*H*, Dr. M. Bird of the same Institute for exposing the flies to it, and Prof. C. M. Stevens, of Pullman, Washington, for sending us a sample of butyl-*H*. Semi-*H*, dissolved in arachis oil in a 5 per cent solution, was applied as aerosol, butyl-*H* as vapour in an air-stream which passed through the