INDUCTION OF MITOTIC CROSSING-OVER IN ASPERGILLUS NIDULANS BY BIFUNCTIONAL ALKYLATING AGENTS

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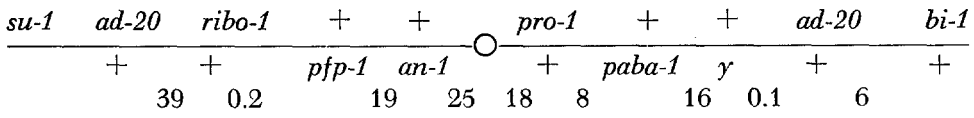
IN previous researches, the ability of various mutagenic agents to induce somatic crossing-over in diploids of *Aspergillus nidulans* has been studied (MORPURGO 1963; STRIGINI, ROSSI and SERMONTI 1963). While X-rays and ultraviolet light and the decay of incorporated ^{32}P produced only a slight increase in crossing-over frequency, methyl-bis(β-chloroethyl) amine (HN-2) induced somatic crossing-over at a very high frequency. These researches have shown that there is no correlation between the mutagenicity of a given agent and its ability in inducing somatic crossing-over and the hypothesis has been advanced that HN-2 is strongly active owing to its bifunctional nature. In the present work, the action of one-armed and two-armed alkylating agents was studied by comparing their activity as mutagens and as inducers of crossing-over.

MATERIALS AND METHODS

The composition of media has already been described (FRATELLO, MORPURGO and SERMONTI 1960). Parafluorophenylalanine (PFP) was added to a final concentration 1 mm into the agar medium. Throughout the present work the diploid strain P was used (*pfp-1*, *an-1*, *paba-1*, *y*; *meth-1*; *nic-8*; *s-12/su-1 ad-20*, *ribo-1*, *pro-1*, *ad-20*, *bi-1*; *acr-1*; *phen-2*; *pyro-4*; *lys-5*). It is prototrophic and fully sensitive to PFP. The genotype of the first linkage group is:



The symbols of PONTECORVO and KÄFER (1958) for mutant alleles were adopted. *pfp-1* means resistance to PFP; *an-1* indicates aneurine (thiamine) requirement.

Detection of segregants: Conidia of strain P plated on minimal medium supplemented with thiamine and PFP cannot grow unless a somatic crossing-over between centromere and *pfp-1*, or a mutation of the wild allele of *pfp-1*, has occurred. Segregants were scored as well-sporulating colonies growing on media supplemented with PFP after plating some thousand diploid conidia.

Detection of mutants: Mutation frequency was measured for resistance to 8-azaguanine in a sensitive haploid strain. The technique has been described by MORPURGO (1962).

Mutagenic treatment: The two-armed methyl-bis(β-chloroethyl)amine (HN-2) and the corresponding one-armed chlorotriethylamine HCl were used in aqueous solution of $NaHCO_3$, in the concentrations respectively of 6.5 mM and 30 mM; the technique has already been described for HN-2 by FRATELLO *et al.* 1960. The two-armed diepoxybutane and the corresponding one-armed ethylene oxide were used in concentrations respectively of 0.02 mM and 2 M in aqueous solution. The treatment with ethylene oxide was carried out at 5°C as its boiling point is about 10°. The other treatments were carried out at room temperature.

RESULTS

Data relative to the lethal and mutagenic effects of the four drugs under consideration are shown in Figure 1. Both one- and two-armed compounds turned out to be powerful mutagens. Data relative to the frequencies of crossing-over, both spontaneous and induced by the four drugs, are given in Figure 2. The data show quite clearly that while one-armed compounds give only a slight stimulation, if any, of this process, both the two-armed compounds produce a strong increase in crossing-over frequencies. The increase induced by HN-2 is especially dramatic, even at levels at which it mutagenic action is not higher than that of the one-armed mustard.

In Figure 2, the ordinate gives the frequency of green, resistant segregants. They have either an^- or an^+ phenotypes. The latter can also be produced by

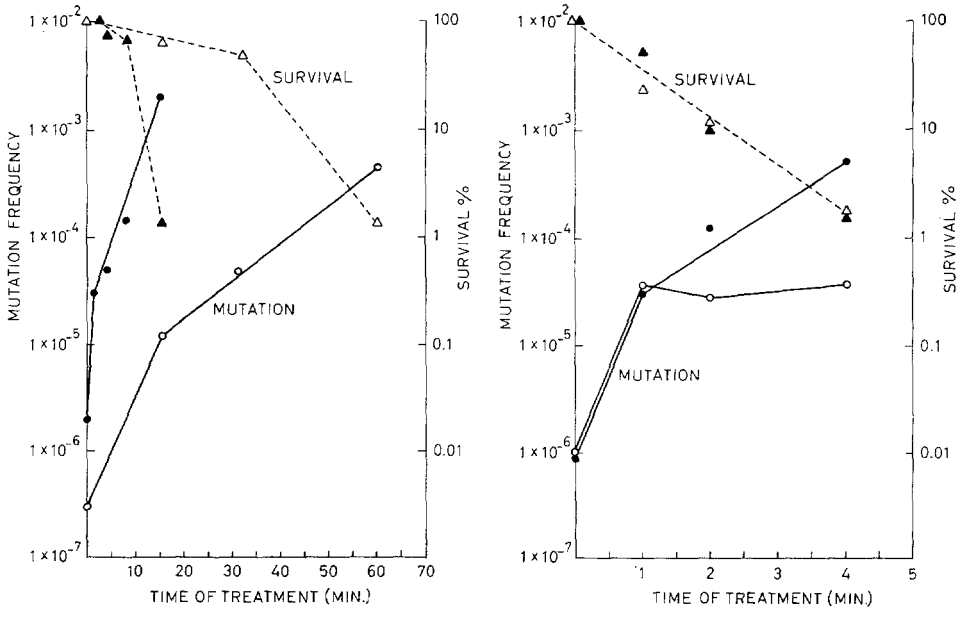


FIGURE 1.—Survival (——) and frequencies of mutation (——) induced by alkylating agents after different periods of treatment. Left—●▲: diepoxybutane; ○△: ethylene oxide. Right—●▲: HN-2; ○△: chlorotriethylamine HCl.

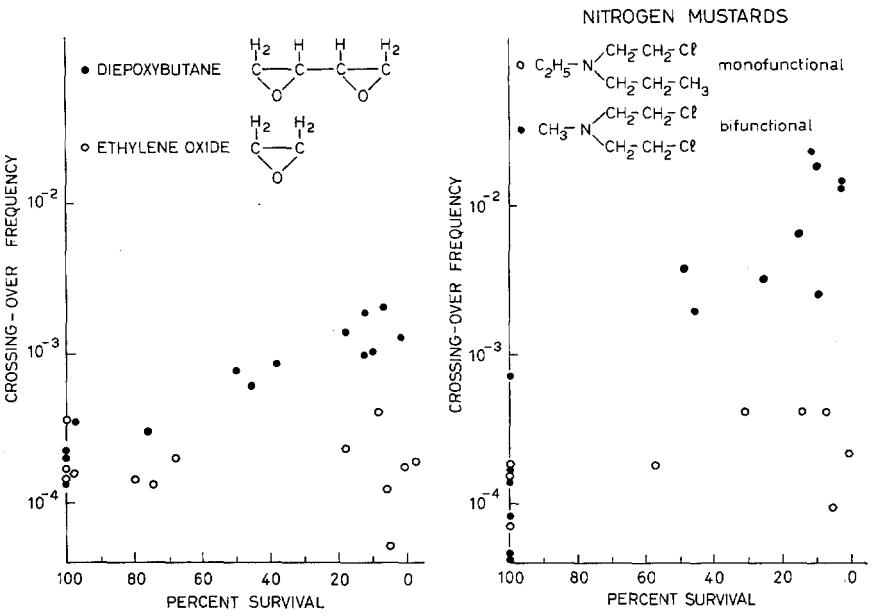


FIGURE 2.—Frequencies of induced crossing-over, following treatment with alkylating agents, at different survivals.

mutation. Since in none of the cases considered did the *an*+ exceed 25% of the total, this distinction was neglected, as it does not cause any important alteration of the data.

The ability of the various compounds in stimulating mitotic crossing-over can be compared at doses which give an approximately equal mutagenic effect. Figure 3 shows the crossing-over frequency after treatment with bifunctional and monofunctional agents, plotted against the mutation frequency.

It is quite clear that at doses giving the same mutation frequency the two armed compounds produce a much higher frequency of crossing-over.

DISCUSSION

The alkylating agents are powerful mutagens independently of their mono- or bifunctional nature (AUERBACH 1958). In *Aspergillus nidulans* the present data show that in the induction of somatic crossing-over the two bifunctional compounds used are much more efficient than the corresponding monofunctional compounds. These molecules have respectively one and two active groups capable of reacting with proteins or nucleic acids. Only the bifunctional agents are able to form intra- or intermolecular cross-links. This higher efficiency could be explained with a mechanism of cross-linkage. The most plausible hypothesis is that the drug establishes a bridge linking two nonsister chromatids, thus providing the condition for a crossing-over to take place. Alkylating agents are able to establish a bridge at the DNA-DNA as well as at the protein-protein level (ALEXANDER

1957). It is not possible at present to speculate as to which of the two possibilities is the correct one.

SUMMARY

The ability of the one-armed alkylating agents ethylene oxide and chlorotriethylamine HCl, and of the two-armed diepoxybutane and methyl-bis(β-chloroethyl)amine, to induce mitotic crossing-over in *Aspergillus nidulans* has been studied with a quantitative technique. At doses which give approximately the same mutagenic effect the two-armed compounds produce a strong increase in the crossing-over frequency while the one-armed compounds are almost ineffective.

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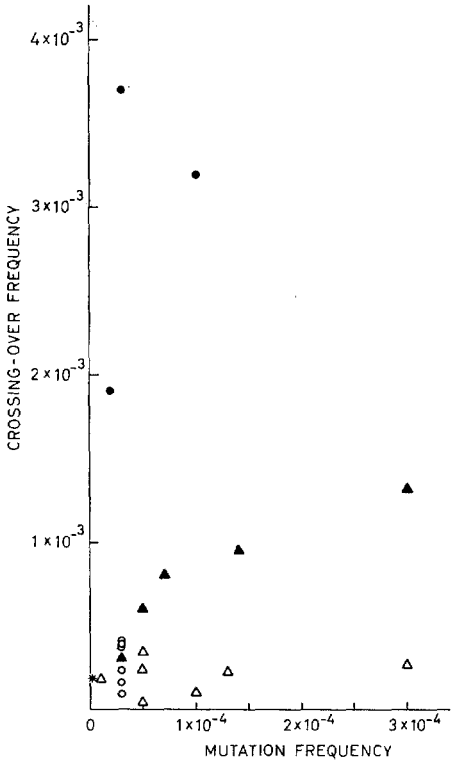

FIGURE 3.—Crossing-over frequencies in diploid strain P plotted against mutation frequencies in haploids at the same dose. ●: HN-2; ○: chlortriethylamine HCl; ▲: diepoxybutane; △: ethylene oxide. The asterisk indicates the average frequency of spontaneous crossing-over and mutation.

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LITERATURE CITED

ALEXANDER, P., S. F. COUSENS, and K. A. STACEY, 1957 The reactions of the mutagenic alkylating agents with proteins and nucleic acid. pp. 294–318; *Ciba Foundation on Drug Resistance in Micro-organisms;* Edited by G. E. WOLSTENHOLME and C. M. O'CONNOR. Churchill, London.

AUERBACH, C., 1958 Mutagenic effects of alkylating agents. Ann. N.Y. Acad. Sci. **68:** 731–736.

FRATELLO, B., G. MORPURGO, and G. SERMONTI, 1960 Induced somatic segregation in *Aspergillus nidulans.* Genetics **45:** 785–800.

MORPURGO, G., 1962 A new method of estimating forward mutation in fungi: resistance to 8-azaguanine and p-fluorophenylalanine. Sci. Repts. Ist. Sup. Sanità **2:** 9–12.

1963 Quantitative measurement of spontaneous and induced somatic segregation in *Aspergillus nidulans.* Sci. Repts. Ist. Sup. Sanità (in press).

PONTECORVO, G., and E. KÄFER, 1958 Genetic analysis based on mitotic recombination. Advan. Genet. **9:** 71–104.

STRIGINI, P., C. ROSSI, and G. SERMONTI, 1963 Effects of disintegration of incorporated ^{32}P in *Aspergillus nidulans.* (In preparation).