SENSITIZATION OF PROTEUS MIRABILIS TO THE LETHAL ACTION OF ETHYL METHANESULPHONATE BY PRETREATMENT WITH MANGANOUS CHLORIDE

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It was reported by Morpurgi and Sermonti (1959) that spores of Penicillium and other microorganisms inactivated by nitrogen mustard may be reactivated by treatment with manganous chloride. In the course of our experiments on the inactivating and mutagenic action of another alkylating agent, ethyl methanesulphonate (EMS), we studied in this connection the influence of pretreatment with $MnCl_2$ on the inactivation of bacteria induced by EMS. With relatively low toxicity EMS has a strong mutagenic effect (Loveless and Howarth 1959 for E. coli and S. typhimurium; Böhme 1961 a for Proteus mirabilis). In the experiments on combined action of EMS and $MnCl_2$ we observed a considerable increase of the toxicity of EMS, if the bacteria were pre-incubated with $MnCl_2$ before treatment with EMS. Figure 1 shows the inactivation curve of the streptomycin dependent strain str-d-3 of P. mirabilis as a function of the incubation time in a 0.25 mol solution of EMS.

After incubation of the bacteria for one hour in $MnCl_2$ solution (3×10^{-3} mol) the EMS induced inactivation rate increases. Under the experimental conditions applied here $MnCl_2$ treatment itself does not have a toxic effect (or only a very slight one)

(Böhme 1961 b). The same effect can be achieved by pretreatment with $CoCl_2$ (Fig. 1).

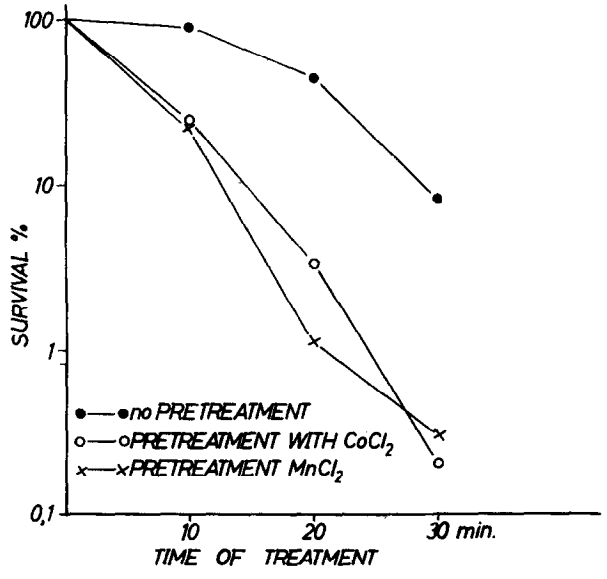

Fig. 1. Inactivation of P. mirabilis strain str-d-3 after treatment with ethyl methanesulphonate (0.25 mol); without pretreatment: a 16 hour culture grown in nutrient broth with 50 γ/ml streptomycin was washed in NaCl (0,9 %) and resuspended in EMS; treatment for the indicated time in a water bath at 37° C; after appropriate dilution into phosphate buffer plating was done on nutrient agar containing 30 γ/ml streptomycin. Pretreatment with $MnCl_2$: after washing the bacteria were resuspended in $MnCl_2$ (3×10^{-3} mol), incubated for 1 hour at 37° C, washed in NaCl (0,9 %) and treated with EMS as above. Pretreatment with $CoCl_2$: the same as above, only pre-incubation with $CoCl_2$ (0,04 %) instead of $MnCl_2$.

Trying to interprete this sensitization effect we considered first as a possible cause the osmotic shock induced by the hypotonic $MnCl_2$ solution, analogous to the effect of $MnCl_2$ treatment and subsequent incubation in citrate buffer observed by Strauss (1961). However, a pretreatment for one hour with distilled water did not sensitize the cells to subsequent EMS treatment. Likewise, the mode of dependence of the sensitization effect on the concentration of the

$MnCl_2$ solution, showing a definite maximum at the concentration of about 3×10^{-3} mol seems to be evidence against the assumption of a change of the permeability induced by an osmotic shock (Fig. 2). Pretreatment of the bacteria with a concentrated $MnCl_2$ solution (3×10^{-1} mol) as well as with solutions below 3×10^{-5} mol do not influence the rate of inactivation induced by subsequent EMS treatment. No change of the sensitivity to EMS was observed after pretreatment of the bacteria with equimolar concentrations of NaCl. However, when the cells were pre-incubated in solutions of $MgCl_2$ we found a decrease of the EMS induced inactivation, the extent of which being dependent on the $MgCl_2$ concentration (the results of these experiments will be presented separately).

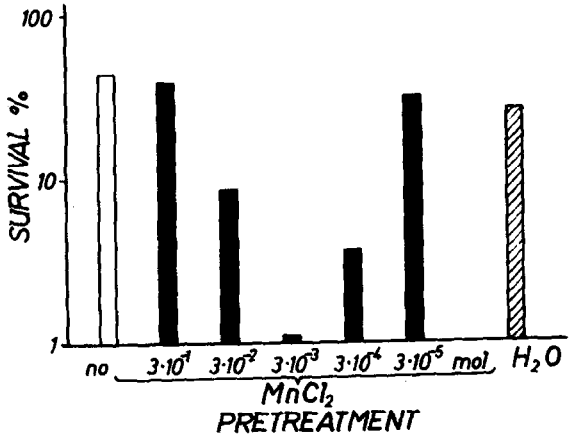

Fig. 2. Inactivation of P. mirabilis strain str-d-3 after treatment with EMS (0,25 mol; 20 min; 37° C) without and with pretreatment with $MnCl_2$ (1 hour; 37° C) of various molarity and distilled water, respectively.

In the concentration range applied in the experiments with Proteus, $MnCl_2$ did not induce a sensitization effect in E. coli B (Loveless pers. comm.) or E. coli K 12 (authors experiments). The sensitization of Proteus by $MnCl_2$ or $CoCl_2$

does not seem to be a general phenomenon with respect to the inactivation induced by all alkylating agents. Whereas a sensitization effect of $MnCl_2$ was found to the lethal action of EMS and in the same way of propane sultone, $MnCl_2$ pre-incubated cells did not show a greater sensitivity to nitrogen mustard than untreated bacteria. The data presented are not yet sufficient to gain a clear understanding of the mechanism of the sensitization effect. Further experiments, especially with simultaneous treatment of the bacteria with $MnCl_2$ and EMS and with posttreatment with $MnCl_2$ are in progress, the results of which will give the possibility to discuss some possible ways of interpretation of the observed facts.

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