NITROFURAZONE AS A MUTAGEN IN ESCHERICHIA COLI

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Mutants of Escherichia coli B or S selected for resistance to any radiomimetic chemical are also resistant to all other radiomimetic chemicals and to ultraviolet and X-radiation (Woody-Karrer and Greenberg, 1963). To the class of radiomimetic agents belong such diverse chemicals as the alkylating agents nitrogen mustard, alkyl-nitrosoguanidines, azaserine and Mitomycin C; nitrofurazone; proflavine and nitrous acid. All these chemicals, with the exception of nitrofurazone (5-nitro-2-furaldehyde semicarbazone), have been shown to be mutagenic for microorganisms. Szybalski (1958) did not find nitrofurazone to be a mutagen for E. coli in a system which measured mutations from streptomycin dependence to independence. However, he found other furazones to be "weak" mutagens in the same system.

In this communication evidence is presented that nitrofurazone is able to induce significant numbers of mutations in E. coli from lactose nonfermenting (Lac^-) to lactose fermenting (Lac^+).

The Lac^- mutants of E. coli strain S were induced by ultraviolet radiation or proflavine. The Lac^- strains were shaken overnight at 37° C in peptone broth[2] washed twice in phosphate-buffered saline,

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[2] Composition of all media used is given in Woody-Karrer and Greenberg (1963)

centrifuged and resuspended in buffered-saline containing nitrofurazone[3] at a concentration of 50 µg/ml. The mixture of cells and chemical, 2 ml in a 50 ml flask, was incubated in a shaking water bath at 37° C. Samples were withdrawn at intervals beginning at time zero and suitable aliquots plated on lactose-salts agar (M9 Lac agar), supplemented with 0.3% peptone broth to allow expression of mutations (Witkin, 1956), and on glucose-salts agar containing 0.3% peptone broth for viable cell count. We and others (Hrishi, 1963) have observed that the number of survivors of E. coli S following treatment with nitrofurazone is dependent on the constitution of the plating medium. However, as seen in Fig. 1 there was essentially no difference in survival over three logarithmic intervals between parent E. coli S (Lac^+) or the mutant Lac^- 1 (see Fig. 2) following nitrofurazone treatment when both were plated on sugar-salts medium containing 0.3% peptone broth. In other experiments the survival of nitrofurazone-treated E. coli strain S was identical when plated on glucose-salts and lactose-salts agar.

Fig. 2 shows that during treatment with nitrofurazone the ratio, R/S, of the number of Lac^+ revertants (R) to the number of surviving bacteria (S) increased as a function of time of exposure. The shape of the curve describing R/S as a function of time of exposure to nitrofurazone was similar for all Lac^- mutants whether induced by ultraviolet radiation or by proflavine. R/S increased most rapidly over the first 30' exposure and after two hours of exposure to nitrofurazone had increased approximately 1000-fold.

In Fig. 3 is shown the survival of E. coli S and Lac^- 1 following ultraviolet radiation and plating on sugar-salts agar with 0.3% peptone broth. There was essentially no difference in survival be-

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[3] A gift from Eaton Laboratories, Division of the Norwich Pharmacal Co., Norwich, N. Y.

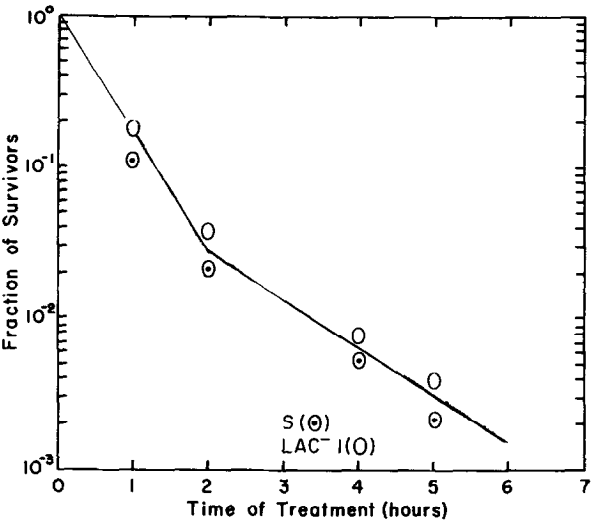

Fig. 1 Survival of E. coli strain S and its mutant Lac⁻ 1 (see Fig. 2) after treatment with nitrofurazone. 10^7 washed, resting cells were suspended in phosphate-buffered saline containing 50 µg nitrofurazone per ml, shaken at 37° C in water bath.

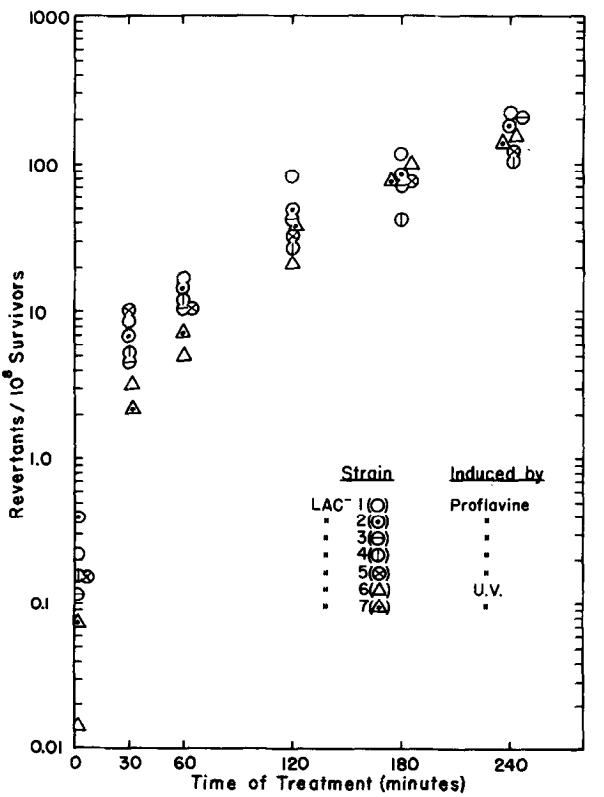

Fig. 2 Ratio of the number of Lac⁺ revertants (R) to the number of surviving bacteria (S) following treatment with nitrofurazone.

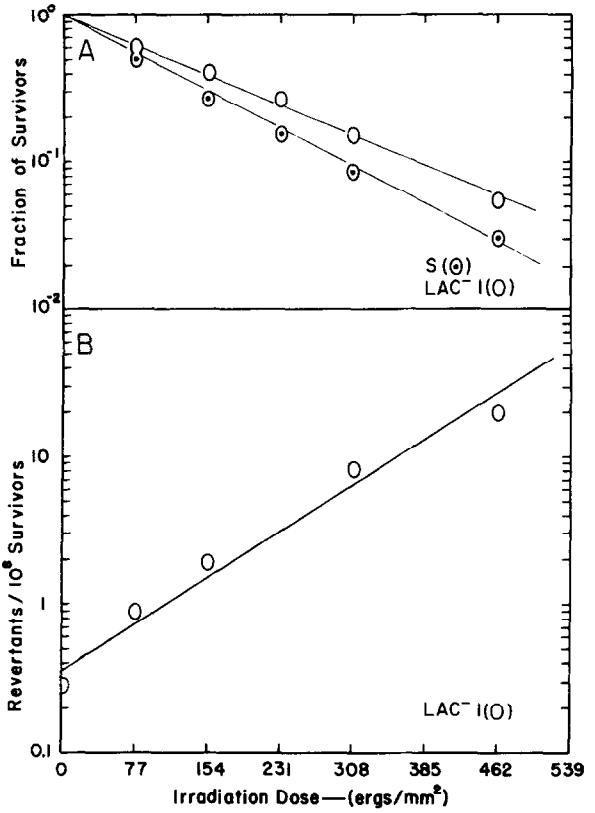

Fig. 3A Survival of E. coli strain S and its mutant, Lac⁻ 1 (Fig. 2), following ultraviolet radiation. Washed, resting cells were irradiated in buffered-saline and plated on sugar-salts agar with 0.3% peptone broth.

Fig. 3B Ratio of Lac⁺ revertants to surviving cells of E. coli S Lac⁻ 1 following ultraviolet irradiation (as in 3A).

tween the two strains. Also shown is the ratio of Lac⁺ revertants to total surviving cells of E. coli S Lac⁻ following treatment with ultraviolet light (λ = 236 mμ). The increase in R/S was approximately 100-fold following an ultraviolet dose of 462 ergs/mm^2.

Nitrofurazone is therefore a potent mutagen in E. coli strain S, able to cause reversions from Lac⁻ to Lac⁺ in a number of Lac⁻ mutants induced by two different mutagenic agents, ultraviolet radiation and proflavine. The general statement that all radiomimetic agents, related to each other by cross-resistance, are mutagens and the reverse is, therefore, supported by the present data. Furthermore, it has

recently been shown (Endo, et al., 1963) that a nitrofuran related to nitrofurazone was able to induce phage maturation in E. coli lysogenic for λ phage, a property shared with ultraviolet radiation, Mitomycin C (Otsuji et al., 1959) azaserine (Gots, Bird and Mudds, 1955) and nitrogen mustard (Lwoff, 1953).

Nitrofurazone is not an alkylating agent, not a deaminating agent, not a photoactivable chemical, nor is it in any obvious way able to cause the formation of thymine dimers. Nitrofurazone offers a potentially useful new tool in the analysis of chemical changes in DNA leading to mutations. Finally, whereas it has not been demonstrated that all clinically useful furazones are mutagenic in bacteria nor that any of them are mutagenic in mammalian cells, the possibility that these therapeutic agents might induce mutations leading to neoplasms should be considered.

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