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SHORT NOTES

Induction of mutation by photodynamic action in *Escherichia coli*

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Photodynamic action, the dye-sensitized autoxidation of the materials accelerated by the action of visible light, is capable of inactivating different kinds of biological systems such as yeast (Freifelder & Uretz, 1960; Uretz & Haynes, 1962), phage (Welsh, 1954; Yamamoto 1958), transforming principles (Bellin & Oster, 1961). Among various photosensitizing agents acridine dyes are of special interest, because these dyes combine specifically with nucleic acids (Lerman, 1963). The photodynamic inactivation of yeast (Freifelder & Uretz, 1960; Uretz & Haynes, 1962) and induction of chromosomal aberration (Kihlman, 1961) with these agents, have been reported. Mutation induction by visible light in *Serratia* pretreated with erythrosine was also reported by Kaplan (Kaplan, 1949). However, photodynamic mutagenecity caused by acridine has not yet been reported so far as the authors know. It is interesting to examine whether or not the action of visible light combined with acridine dyes can alter genetic material and induce mutation.

Tryptophan requiring strain of *Escherichia coli* K12 (λ^-); W3623 was used in the present experiments. The resting cells were diluted to a concentration of 10^9/ml. by Tris buffer solution (pH 7) containing different concentrations of acridine. Two millilitres of the cell suspensions were irradiated by visible light in a glass test tube. The source of the light was a 500-watt flood lamp with a filter of water 5 cm. in thickness. In the bulk of the experiments sufficient quantity of air was supplied by bubbling during the irradiations. Noticeable temperature rise was observed in none of the samples. Mutation was measured by reversion of tryptophan dependence to independence.

If the photodynamic action can induce mutations, it should be expected that the mutation frequency increases with increase of incident energy of the visible light. Figure 1 shows experimental curves of mutation frequency versus exposure time under aerobic and anaerobic conditions for acridine orange at the concentration of 50 μg./ml. Treatment with acridine only, without exposure to the light, did not enhance the mutation frequency compared with controls. But when it was combined with visible light, the mutation frequency was remarkably enhanced with the increase of exposure time. In reconstruction experiments the tryptophan independent cells showed a little selective advantage over the tryptophan dependent cells in surviving the photodynamic inactivation. This advantage, however, was not so great as to explain the observed increase in mutation frequency. In fact, under the appropriate experimental conditions, the number of mutants per plate was ten times as large or more under photodynamic action than in the controls. Therefore, it is concluded that the photodynamic action or acridine dye actually can induce mutations.

The mutation frequency curve as a function of incident energy of visible light is of a multi-hit type (*ca.* 2·6 hit response). A similar pattern has always been obtained in repeated experiments. The multiplicity for mutation induction is of same order as for killing, as can be seen from the curve of survival versus exposure time (Fig. 2).

It has been generally accepted that oxidation plays an important role in photodynamic

inactivation processes. To estimate the effect of oxidation on the mutation induction by photodynamic action, the anoxia experiments were carried out by replacing air with nitrogen. In Fig. 1 it is shown that the mutagenic activity of photodynamic action was markedly decreased under anoxia. Thus, it may be concluded that oxidation plays an important role in photodynamic action for both mutation induction and killing.

The effect of the concentration of the acridine orange upon the mutation induction is shown in Fig. 3. At low concentrations, the mutagenic activity was directly proportional to the concentration of the dye, probably because the amount of dye combined with DNA

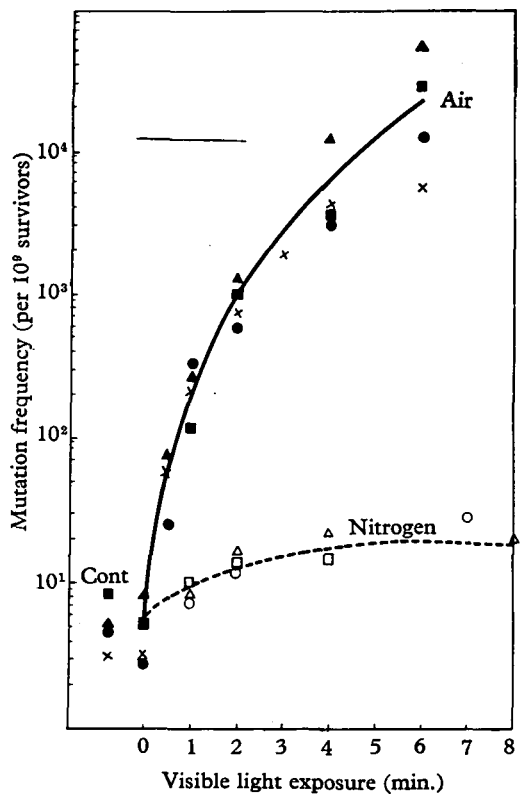

Fig. 1, Effect of visible light exposure on the induction of mutation in dye-sensitized *E. coli*.
Dye concentration: 50 μg./ml. of acridine orange.
Air: bubbling of air.
Nitrogen: bubbling of nitrogen.
Cont.: no dye supplement and no light exposure.

is one of the main rate-limiting factors. The amount of the dye absorbed to genetic materials or nucleic acids is proportional to its concentration. At higher concentrations, however, mutagenic activity would reach saturation.

The derivatives of acridine dyes were tested for photodynamic mutagenic activity. Strong mutagenic action was revealed in acridine orange, acridine yellow, methylene blue and toluidine blue. But, under our experimental conditions, acridine red did not show any measurable mutagenic activity. Suzuki (personal communication) has recently found that interaction of acridine red with DNA is very weak in comparison with acridine orange or yellow. Thus, it might be supposed that the binding of DNA with dye is an important factor for the induction of mutation by photodynamic action.

Our experiments have clearly shown that a certain type of photodynamic action can induce mutations, and that the action requires: (*a*) combining of the dye with the genetic materials, (*b*) the presence of oxygen and (*c*) sufficient energy of visible light absorbed by the complex of dye and genetic materials.

This induction of mutation by photodynamic effect is probably due to the photoxidation of genetic materials (DNA). The mechanism of induction of mutation by photodynamic action may differ from that suggested by Brenner *et al.* (1961), for phage mutation induced by acridine only, since no mutation could be induced without visible light exposure

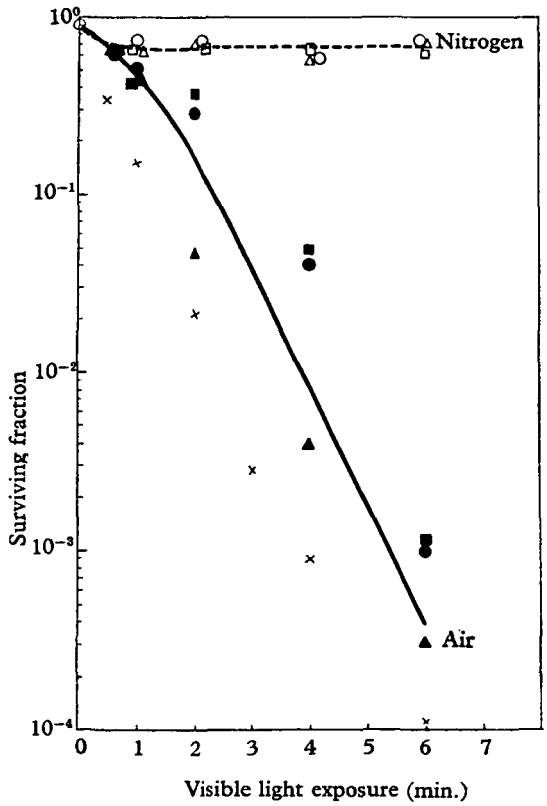

Fig. 2. Effect of visible light exposure on the inactivation of dye-sensitized *E. coli*. Experimental conditions and abbreviations are the same as in Fig. 1.

in our experiments. When photosensitizing dyes bind with genetic materials, the exposure to visible light would produce certain reactions mediated by oxidation either within or in the vicinity of the gene. These reactions will initiate the destruction of the genetic materials and eventually lead to mutation. Freifelder *et al.* (1961) reported that the photodynamic action of DNA *in vitro* produces the depolymerization of DNA, apparently through single strand scission, although some double strand scission may not be excluded. The detailed mechanism of photodynamic mutagenicity remains obscure. However, it may be assumed that mutation is largely of the deletion type caused by DNA strand scission, because the experimental results given in Fig. 1 show a two-to-three hit response for mutation induction.

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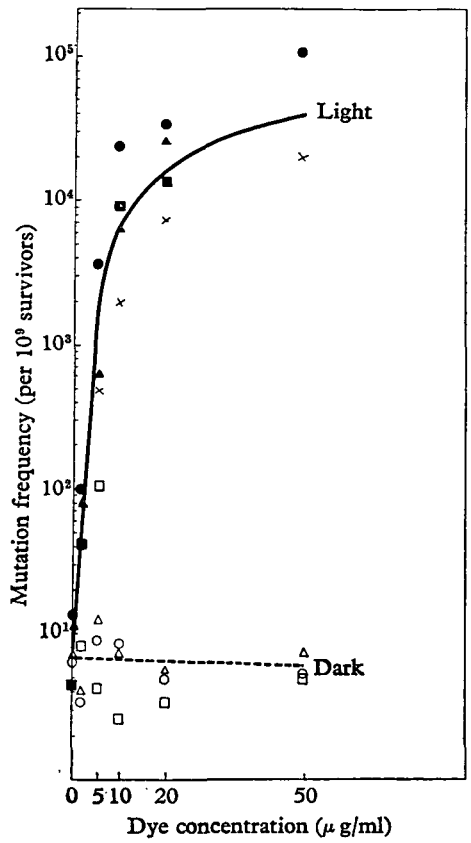

Fig. 3. Effect of dye concentration on induction of mutation by photodynamic action in *E. Coli*. Light exposure time is 6 min.

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