MUTAGENIC AND ANTIMUTAGENIC EFFECTS OF ACRIDINE ORANGE IN ESCHERICHIA COLI[1]

R. B. Webb and H. E. Kubitschek

Division of Biological and Medical Research
Argonne National Laboratory
Argonne, Illinois

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The acridines are known to be good mutagens for the T-even bacteriophages (Orgel and Brenner, 1961). In bacteria, a mutagenic effect has been reported for acriflavine at concentrations which kill most of the cells (Witkin, 1947), but the acridines have been without noticeable effect in "vegetative" bacteria (Lerman, 1963). Stimulated by Lerman's evidence (1961) for the intercalation of acridines in DNA, Brenner et al. (1961) proposed that the presence of the acridines leads to mutation by insertions or deletions of base pairs in DNA.

We have examined the effect of acridine orange (AO) on mutation rates in continuous (chemostat) cultures of Escherichia coli B/r/1,try to resistance to bacteriophage T5. There was no observable cell death. In the presence of light AO is a very effective mutagen. In the absence of light, mutation rates appear to be lower than the spontaneous mutation rates; that is, in the dark AO is an antimutagen. These results might be expected on the basis of in vitro measurements of AO-DNA complexes by Freifelder et al. (1961), who found that DNA was degraded in the presence of AO and oxygen in the light, but not in the dark. They also found an increase in the thermal denaturation temperature of

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DNA in the presence of AO. Since one hypothesis of the mutagenic action of caffeine is that caffeine lowers the thermal denaturation temperature of DNA, we thought that AO might be antimutagenic for caffeine. This result is found.

EXPERIMENTAL

Continuous cultures of *E. coli* B/r/1,try$^-$ were grown in a minimal medium containing tryptophan and limited with glucose, and cells were counted by methods reported previously (Kubitschek and Bendigkeit, 1961). AO was added to concentrations from 10^{-6} M to 5×10^{-6} M. When caffeine was used as the mutagen, it was added to give a concentration of 0.45 g/l (2.3×10^{-3} M). For dark experiments the chemostats were placed in a light shield, and in some cases also in a darkened room. In other experiments, light was supplied by a 4-watt, white fluorescent lamp placed about 10 cm from the growth tube. Light intensities were measured with a Westinghouse illumination meter.

At a concentration of 10^{-6} M AO, a mutation rate of about 1×10^{-8} mutants per day per bacterium per ft-candle is found (Fig. 1). The initial part of the curve in Fig. 1 also shows that in the absence of light the mutation rate with AO is low. Spontaneous mutation rates in glucose-limited cultures, which appear to be directly proportional to the generation rate of the culture (Kubitschek and Bendigkeit, in preparation), are larger than those for light-shielded chemostats containing AO. It is quite possible that mutation rates with AO are too large because of inadequate light shielding; even a minute amount of stray light, less than one ft-candle, might be expected to increase the mutation rate. This would also explain the rather lower rate for a chemostat which was both shielded and placed in a darkened room. Nevertheless, AO reduces the mutation rate significantly.

Table I also shows that caffeine-induced mutation rates are reduced in the presence of AO, and by about the same factor found for spontaneous mutation.

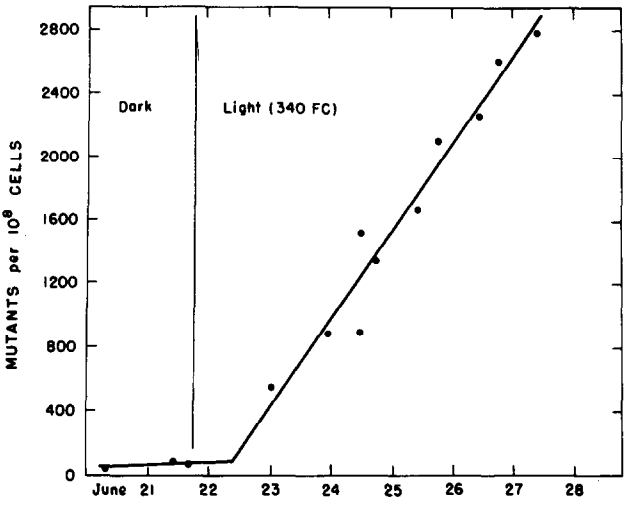

Figure 1. Daily accumulation of T5-resistant mutants of E. coli B/r/1,try⁻ in a glucose-limited (80 mg/l) chemostat containing 10^{-6} M AO exposed to light after an initial period of growth in the dark.

Table I

Mutation rates in light-shielded chemostats

	Divisions per day	Mutants/div/10^8 bact
Spontaneous, no AO	2-7	4.6*
AO		
10^{-6} M	3.9	2.6
2 x 10^{-6} M	4.7	1.5**
2.5 x 10^{-6} M	6.2	1.5
5 x 10^{-6} M	6.9	3.7
Caffeine, no AO	4.6	31**
Caffeine + 2 x 10^{-6} M AO	5.2	9**

* Data of Kubitschek and Bendigkeit (unpublished)

** Light-shielded chemostat in darkened room

DISCUSSION

The above results do not support the intercalation model of mutagenesis proposed by Lerman (1961), since this model requires that AO be mutagenic in the dark. However, if intercalation is the primary mode of binding of acridine to DNA, then intercalation can reasonably be expected to be responsible for the antimutagenic action of AO reported here.

It is difficult to reconcile the antimutagenic action of AO in our experiments with the potent mutagenicity of acridines for the T-even phages in the dark (Lerman, 1963). Lerman suggested that the explanation might lie in the extensive genetic recombination accompanying phage multiplication. In view of the extremely active photodynamic action of AO, however, we do not wish to adopt this viewpoint prematurely.

Our results support a different interpretation of the antimutagenesis of AO in the absence of light. This view follows rather naturally from a proposal by one of us (HEK, in preparation) for the mechanism of mutagenic action of caffeine: caffeine serves to decrease the denaturation temperature of DNA within the cell, increasing localized melting of strands, and thereby facilitating increased mutation from natural causes. This interpretation would predict that a compound which raises the denaturation temperature of DNA might be expected to act as an antimutagen. Thus the finding by Freifelder et al. that AO increases the denaturation temperature of DNA strands (albeit with measurements made at low ionic strength) suggested that AO might be antimutagenic both for spontaneous mutation and for caffeine, as is found.

Two alternative mechanisms of the antimutagenic action of AO upon caffeine can be proposed: AO may form a complex with caffeine and thereby reduce its effectiveness or AO and caffeine may bind compet-

itively to DNA. However, these mechanisms fail to provide a direct explanation for the antimutagenicity of AO in the absence of caffeine.

Depolymerization of DNA and destabilization of secondary structure by AO in the presence of oxygen are presumably due to oxidation of deoxyribose or a base (Freifelder et al., 1961). These reactions might be involved in photodynamic mutagenesis. On the basis of Lerman's evidence, it is an attractive possibility that both photodynamic mutagenesis and antimutagenesis of AO in the dark could arise as a result of intercalation.

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