THE ELIMINATION OF F'lac$^+$ FROM ESCHERICHIA COLI BY MUTAGENIC AGENTS

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Acridine dyes have been used extensively to eliminate various extra-chromosomal genetic elements such as the sex factor F (Hirota, 1960), F merogenotes (Adelberg and Burns, 1960), ColV (Kahn and Helinski, 1964), and R factors (Mitsuhashi et al., 1961) from bacterial cells. The efficiency varies from less than 1% to more than 99% depending upon the element involved, the bacterial strain, and the conditions used.

Hirota (1960), and later Jacob, Brenner, and Cuzin (1963), suggested that a possible mechanism was that the F replication system is susceptible to concentrations of acridines not affecting the chromosomal replication system.

It is known that acridines are potent mutagens for the bacteriophage T4 (Orgel and Brenner, 1961), even though the evidence for a mutagenic effect on chromosomal genes is contradictory (see Zampieri and Greenberg, 1965). Thus another mechanism for episome elimination might be production of mutations in the genes controlling replication in the episome. In this paper it is shown that various known mutagenic agents are able to eliminate an F merogenote from E. coli.

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Abbreviations: acridine orange (AO), N-methyl-N'-nitro-N-nitrosoguanidine (NMG), ethyl methane sulphonate (EMS), ultraviolet light (UV).

Materials and Methods. E. coli K12 (F*lac*$^+$) Lac$^-$ Sm^R Thi$^-$ (NW3) was obtained by crossing E. coli K12 W1655 (F*lac*$^+$) Lac$^-$ Sm^S Met$^-$ with E. coli K12 C600 F$^-$ Lac$^-$ Sm^R Thr$^-$ Leu$^-$ Thi$^-$.

Cultures were grown with shaking in the dark at 35°C in nutrient broth (Difco nutrient broth powder, 12 g/L; Difco yeast extract, 3 g/L) adjusted to the required pH. The buffer solution used contained KH_2PO_4, 3 g/L; Na_2HPO_4, 7 g/L; NaCl 4 g/L; $MgSO_4$ 7 H_2O, 0.2 g/L.

Lac$^-$ colonies were identified by plating on EMB-lactose agar (Lederberg, 1950), containing 200 µg/ml streptomycin.

Lac$^-$ colonies were tested for maleness with the male-specific phage MS2, by adding 1 ml of a suspension of 250 phage/ml in top agar (Brinton et al., 1964), to 0.1 ml of an overnight culture, and incubating overnight to allow development of plaques.

The Lac$^-$ MS2-resistant colonies were further tested by mating with the original W1655 (F*lac*$^+$) strain, and plating on EMB-lactose-streptomycin agar to determine the efficiency of reinfection by F*lac*$^+$.

Results. The effects of NMG and EMS on growing cells was tested using concentrations of the mutagens which allowed growth of a culture inoculated with 10^4 cells/ml. The results of three typical experiments are given in Table 1. Both led to the production of a substantial proportion of Lac$^-$ (cured) cells, with an efficiency varying somewhat with the pH and the concentration of mutagen used.

Time course experiments were then performed, using the conditions giving the best curing (Fig. 1). The kinetics of curing by EMS and NMG differed both from each other and from curing by AO.

It would be expected that if curing took place by a mechanism involving mutagenesis rather than replication, then it should occur when non-growing cells are treated. This was tested by resuspending exponentially-growing cells in buffer at pH 7.0 (Fig. 2). Both NMG and EMS had a noticeable effect, whereas AO did not. Similar results were obtained by repeating the experiments

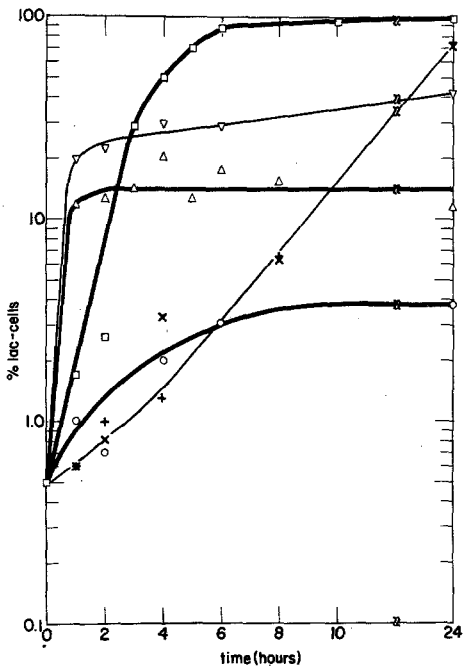

Fig. 1. The elimination of Flac$^+$ from growing cells. Cultures were inoculated with 10^4 cells/ml and incubated in the dark. Samples were removed periodically for determination of the percentage of Lac$^-$ cells. Symbols: 0, control; □, 25 μg/ml AO, pH 7.6; △, 100 μg/ml NMG, pH 7.6; ▽, 50 μg/ml NMG, pH 7.0; x, 0.02 M EMS, pH 7.6; +, 0.01 M EMS, pH 7.0.

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at pH 7.6.

The effect of UV was also tested on non-growing cells (Table 2). Irradiation to 0.1% survival or less left a population of cells in which about 10% were Lac$^-$.

In order to correlate production of Lac$^-$ cells with true episome elimination, Lac$^-$ colonies picked at random from various experiments were shown to have become resistant to MS2 phage after curing with AO (40/40 colonies tested), UV (42/42), NMG (80/80), or EMS (75/80). In addition, 20 of each of these sets of Lac$^-$ MS2-resistant cells could be reinfected with Flac$^+$ at the high efficiency expected for F$^-$ strains.

Table 1

The Elimination of F*lac*$^+$ from Growing Cells

Addition	Concentration	pH	% Lac$^-$ Cells Experiment 1	2	3
-	-	7.0	6	7	1
AO	25 µg/ml	"	0	33	54
NMG	50 "	"	75	48	72
EMS	0.01 M	"	88	77	55
EMS	0.02 "	"	25	20	-
-	-	7.6	2	0	7
AO	25 µg/ml	"	99	98	100
NMG	50 "	"	11	5	15
NMG	100 "	"	27	14	15
EMS	0.01 M	"	49	12	17
EMS	0.02 M	"	88	81	69

Cultures were inoculated with 10^4 cells/ml taken from an exponentially-growing culture, and incubated in the dark for 24 hrs. Dilutions were plated on EMB-lactose-streptomycin to determine the percentage of Lac$^-$ cells.

Table 2

The Elimination of F*lac*$^+$ by UV

Time of Irradiation (sec)	Average Survival (%)	% Lac$^-$ Cells Experiment 1	2	3
0	100	0.3	0.6	0.4
15	25	0.2	7.5	-
30	2	3.5	7.7	8.2
45	0.1	5.5	11.8	8.3
60	0.01	6.0	10.7	11.4
75	0.001	-	8.8	11.1

Exponentially-growing cells were washed, resuspended in saline at 10^8 cells/ml and irradiated with a 15 w. G. E. germicidal lamp placed 35 cm away. Samples were removed periodically for determination of survival and the percentage of Lac$^-$ cells.

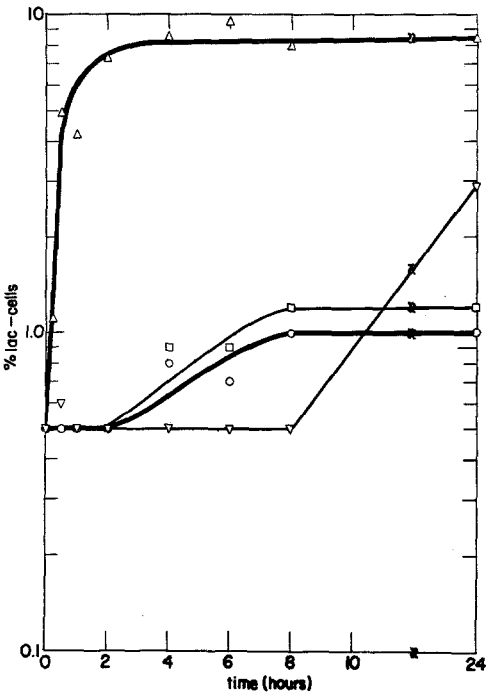

Fig. 2. The elimination of F<u>lac</u>$^+$ from non-growing cells. Exponentially-growing cells were washed and resuspended in buffer, pH 7.0, at 10^8 cells/ml, and incubated in the dark. Samples were removed periodically for determination of the percentage of Lac$^-$ cells. Symbols: 0, control; □, 25 μg/ml AO; △, 100 μg/ml NMG; ▽, 0.02 M EMS.

<u>Discussion</u>. The results show that both EMS and NMG are effective agents for elimination of the episome F<u>lac</u>$^+$ in growing cultures. Loss of the Lac$^+$ phenotype was always due to true loss of the episome, except in the case of EMS, when 5 of the 80 colonies tested were still sensitive to MS2 phage. These five transferred chromosomal markers at high frequency, and were thus presumably (FLac$^-$) strains (Willetts, unpublished results). The differences in the time courses of curing by AO, EMS, or NMG could have been due to several factors - difference in the stabilities of the mutagens, (leading to different effective periods of action), initial growth inhibition and cell killing, and transfer of the episome back to cured cells. All of

these factors were different for the three mutagens (Willetts, unpublished results).

These results, and especially the elimination produced by UV, NMG, and EMS in non-growing cell suspensions suggest that a mechanism involving mutagenesis may, in fact, lead to elimination of F*lac*$^+$. Failure to cure non-growing cells shows that cell growth is essential for curing by AO, although a mechanism involving mutagenesis can still not be eliminated since replication is probably necessary for the production of mutations by AO (Brenner *et al.*, 1961).

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