THE ELIMINATION BY ACRIDINE ORANGE OF F30 FROM RECOMBINATION-DEFICIENT STRAINS OF *ESCHERICHIA COLI* K12¹

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LOSS of an F-merogenote from an F-merodiploid cell occurs at a low frequency during vegetative cell growth, but this frequency can be greatly increased by growing the cells in broth at a high pH in the presence of an acridine dye ("curing," HIROTA 1960). This effect could conceivably be due to acridine interfering either with the correct distribution of the F-merogenote into the daughter cells at cell division, or with the multiplication of the F-merogenote. HIROTA (1960), STOUTHAMER, DE HAAN, and BULTEN (1963), and JACOB, BRENNER, and CUZIN (1963) have suggested that multiplication of the F-merogenote relative to the chromosome is decreased due to a greater inhibition of the F replication system than of cell growth.

CLOWES and MOODY (1967) suggested that recombination is necessary for curing by acridine orange (AO), since curable sex factors can recombine with the chromosome in Rec⁺ strains giving efficient chromosome mobilization, whereas non-curable ones cannot, and their inefficient transfer of the chromosome is not further depressed in Rec⁻ strains (CLOWES and MOODY 1966). Also, both they and SESNOWITZ (personal communication) found that the sex factor F cannot be cured from a particular Rec⁻ strain by AO. In addition, a parallel is drawn with the mutagenic action of acridines which is only apparent in bacteria under conditions where recombination can occur (SESNOWITZ, ADELBERG, and BOYER 1966).

The present work involves an investigation of the F30 derivatives of different types of Rec⁻ strains derived from two different parental strains. CLARK (1967) has shown that Rec⁻ strains may be divided into three phenotypic groups. Rec₁⁻ strains are about 100 times more ultraviolet (UV) sensitive than the wild type, the level of spontaneous lambda production by lysogenic derivatives is normal, and fertility with the Hfr strain KL98 is reduced about 50-fold. Rec₂⁻ and Rec₃⁻ strains are about 10⁴ times more UV sensitive than the wild type, the level of spontaneous lambda production by lysogenic derivatives is greatly depressed, and their fertility with KL98 is reduced by a factor of about 10⁴; they differ in their fertility with the Hfr strain KL16, Rec₃⁻ strains being noticeably less fertile. AO curing of the F30 merodiploids was measured, to see if a fully-active recom-

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ination system is mandatory for curing, or if differences between the three phenotypic Rec⁻ groups could be detected. AO sensitivity was followed simultaneously, and the relationship of this to the Rec⁻ phenotype and the degree of curing is discussed. During these experiments, clones having the properties of neither the merodiploid nor the F⁻ parental strain were found, and some evidence is presented concerning their derivation.

MATERIALS AND METHODS

Bacterial strains: The properties of the strains used are listed in Tables 1 and 2. F30 was derived from the F merodiploid strain G1, constructed by DR. E. P. GOLDSCHMIDT and kindly provided by DR. T. S. MATNEY. It carries several wild type genes of the *his* operon, except for the presence of a *his-323* mutation, but carries no other known genes. The *his* genes carried by F30 complement *his-1* in JC411, *his-4* in AB1157, and *his-504* in JC2444. F30 is a "normal" F prime in that it mobilizes chromosomal markers at good frequencies (BASTARRACHEA and CLARK, in preparation). The proposal of MATNEY, GOLDSCHMIDT, and MANDEL (1966) that F30 carries a *rec+* allele has now been shown to be incorrect both by the original authors and in this laboratory.

The F30 merodiploid strains were constructed by mating the F30 donor JC4046 with JC411 or AB1157 or their Rec⁻ derivatives. A tenfold excess of the female strain was used in a 60 minute mating in L broth, and His⁺[Sm^R] conjugants were selected. The frequencies of formation of His⁺ conjugants by the Rec⁻ strains relative to the Rec⁺ parental strains are shown in Table 2, and are similar to those found by CLARK (1967) for the formation of Lac⁺ conjugants using E3 as donor strain. One merodiploid from each cross was purified and tested for its UV sensitivity and growth requirements before use in the experiments described below.

Flac⁺ merodiploid derivatives of some of the Rec⁺ and Rec⁻ strains were constructed similarly, using the strain E3 as donor, and selecting for Lac⁺[Sm^R] conjugants.

Media: Media have been described previously by CLARK (1967). Preliminary experiments showed that pH 7.8 was necessary for maximal curing of JC5414 by 50 µg/ml AO, and accordingly, L broth adjusted to pH 7.80-7.90 with NaOH was used in all of the AO curing and sensitivity experiments.

TABLE 1
Characteristics of bacterial strains

Strain	Sex	Rec	Ade	Arg	His	Lac	Leu	Met	Str	Thi	Thr	Try
JC411	F ⁻	+	+	—	<i>his-1</i>	—	—	—	R	+	+	+
AB1157	F ⁻	+	+	—	<i>his-4</i>	—	—	+	R	—	—	+
JC4046	F30	+	+	+	+	—	+	+	S	+	+	+
JC2444	F ⁻	+	+	+	<i>his-504</i>	—	+	+	S	+	+	+
JC182*	Hfr	+	—	+	+	+	+	+	S	—	+	+
E3	<i>Flac</i> ⁺	+	+	+	+	+	+	+	S	—	+	+
JC5492†	<i>Flac</i> ⁺	+	—	—	+	+	+	+	R	—	+	—
JC5493‡	<i>Flac</i> ⁺	<i>rec-12</i>	—	—	+	+	+	+	R	—	+	—
JC5494‡	<i>Flac</i> ⁺	<i>recA13</i>	—	—	+	+	+	+	R	—	+	—
JC5498‡	<i>Flac</i> ⁺	+	+	—	<i>his-4</i>	+	—	+	R	—	—	+
JC5499§	<i>Flac</i> ⁺	+	+	—	<i>his-4</i>	+	—	+	R	—	—	+

Genetic symbols are those used by TAYLOR and TROTTER (1967).

* See CLARK 1963.

† These are *Flac*⁺ derivatives of CysC⁺ cotransductants derived from AT1378 (B. Low, N. S. WILLETTS, and A. J. CLARK. To be published.)

‡ This is an *Flac*⁺ derivative of a UV^R Rec⁺ revertant of AB2462.

§ This is an *Flac*⁺ derivative of a UV^R Rec⁺ transductant of AB2463.

TABLE 2
Characteristics of the F30 derivatives

Strain	F ⁻ parent	<i>rec</i> allele	Recombination phenotype	Reciprocal of relative frequency of F30 merodiploid formation
1. <i>Derivatives of JC411</i>				
JC4087	JC411	<i>rec</i> ⁺	Rec ⁺	1
JC4086	JC1553	<i>recA1</i>	Rec ₂ ⁻	3
JC4100	JC1554	<i>rec-2</i>	Rec ₂ ⁻	3
JC4101	JC4422	<i>rec-62</i>	Rec ₂ ⁻	3
JC4102	JC4423	<i>rec-70</i>	Rec ₃ ⁻	3
JC4103	JC4535	<i>recB60</i>	Rec ₁ ⁻	5
JC4104	JC4536	<i>recB61</i>	Rec ₁ ⁻	5
2. <i>Derivatives of AB1157</i>				
JC5414	AB1157	<i>rec</i> ⁺	Rec ⁺	1
JC5415	AB2462	<i>rec-12</i>	Rec ₃ ⁻	3
JC5416	AB2463	<i>recA13</i>	Rec ₂ ⁻	9
JC5417	AB2470	<i>recB21</i>	Rec ₁ ⁻	9
JC5418	AB2690	<i>rec-65</i>	Rec ₃ ⁻	2
JC5419	JC2761	<i>rec-56</i>	Rec ₂ ⁻	3
JC5420	JC4457	<i>recB58</i>	Rec ₁ ⁻	13

The phenotypic recombination groups are described by CLARK (1967). The determination of the frequency of F30 merodiploid formation is described under MATERIALS AND METHODS, and these are compared to the values obtained for the parental Rec⁺ strains. The four Rec₁⁻ strains have all been shown to be mutants in *recB* by complementation tests performed in this laboratory. (WILLETTS and MOUNT, to be published)

Mating conditions and ultraviolet sensitivity tests: These have been described previously by CLARK and MARGULIES (1965).

MS2 phage sensitivity: This was done as described by WILLETTS (1967).

Acriflavin uptake: This was measured according to the method of SILVER and WENDT (1967).

AO treatment. An inoculum of 5×10^3 – 5×10^4 cells/ml taken from an overnight standing culture in L broth pH 7 was added to 2 or 4 ml of L broth pH 7.80–7.90 containing various concentrations of AO. The tubes were covered with aluminum foil to exclude light, and shaken for 20 hours at 37°. Suitably diluted samples were plated on L plates which were incubated overnight. The plates were counted to determine the extent of growth inhibition and 100 single colonies derived from each tube were patched onto L plates which were then incubated for 6 or 16 hours.

These master plates were replicated onto supplemented minimal medium plates with and without histidine in order to determine the proportion of cells which had lost the F-merogenote. They were also replicated on a lawn of the His⁻ female strain JC2444, spread on minimal medium plates selective for His⁺ prototrophic conjugants, in order to correlate the His⁺ phenotype with transfer ability.

RESULTS

Sensitivity to AO: The plate counts read after the AO treatment described above gave a measure of the AO sensitivity of the strains. These results are presented in detail in Figures 1 and 2, and for ease of comparison, data taken from these figures are presented in Table 3. The inhibition ratio is a complex measure of the response of the cell to AO. Growth inhibition, bacteriostatic, and

FIGURE 1.—The AO sensitivity of the F30 merodiploid strains derived from JC411. The “inhibition ratio” is the ratio of the cell titre found after incubation in the absence of AO ($1-2 \times 10^9$ /ml) to the cell titre found after treatment with a given concentration of AO.

bactericidal effects may all be involved, but no attempt was made to distinguish their separate contributions in these experiments.

The most striking difference found was between the AO sensitivities of Rec^+ and Rec^- strains: the Rec^- strains were all much more sensitive.

Some correlation between the UV and AO sensitivities of the different phenotypic Rec^- groups was also looked for, since Rec_1^- strains are much more resistant to UV than Rec_2^- and Rec_3^- . However although the Rec_1^- strains derived from JC411 were more resistant to AO than the Rec_2^- and Rec_3^- strains, the Rec_1^- ,

FIGURE 2.—The AO sensitivity of the F30 merodiploid strains derived from AB1157. Results expressed as in Figure 1.

Rec_2^- , and Rec_3^- strains derived from AB1157 were all equally sensitive. Examination of more strains would be necessary to establish any correlation between UV and AO sensitivity of Rec^- strains derived from either of these parental strains. Of course, the sensitivities are based on two different criteria, on growth inhibition, bacteriostatic, and bactericidal effects for AO, and for cell killing by UV, and may not be strictly comparable.

Since Rec^- strains were all more sensitive to AO than Rec^+ , it seemed possible that Rec^+ revertants of the Rec^- strains might have been selected during the AO

TABLE 3

The relationship of the recombination phenotype to curing and growth inhibition by AO

Strain	Phenotypic Rec group	25 µg/ml AO		Inhibition ratio=10 ³	
		Percent curing	Inhibition ratio	Concentration of AO (µg/ml)	Percent curing

<i>Derivatives of JC411:</i>					
JC4087	+	92	1 × 10 ⁰	91	95
JC4103	1	99	1 × 10 ²	26	100
JC4104	1	96	4 × 10 ¹	32	87
JC4086	2	100	6 × 10 ³	21	96
JC4100	2	92	4 × 10 ⁵	12	63
JC4101	2	..		1	0
JC4102	3	95	4 × 10 ³	22	95
<i>Derivatives of AB1157:</i>					
JC5414	+	92	1 × 10 ⁰	79	<20
JC5417	1	96	6 × 10 ⁴	18	58
JC5420	1	17	1 × 10 ⁴	20	17
JC5416	2	0	2 × 10 ⁴	18	0
JC5419	2	29	6 × 10 ³	22	28
JC5415	3	3	3 × 10 ¹	40	0
JC5418	3	9	1 × 10 ⁴	20	7

These data are taken from Figures 1-4.

treatment. Therefore, the master plates prepared as described in the preceding Section were replicated onto L plates to test the UV sensitivity of the survivors, and onto a lawn of the donor strain JC182 spread on supplemented minimal medium plates selective for Arg⁺ [Ade⁺Sm^R] recombinants, to test their recipient and recombination ability. JC4087 and JC5414 both gave rise to such recombinants with JC182, even though they are F merodiploids, whereas the Rec⁻ F30 derivatives did not. In only one case, of JC5416 treated with 25 µg/ml AO, were UV^R Rec⁺ revertants found (3%). In all other cases, the survivors had retained their UV^S Rec⁻ phenotype.

The AO sensitivities of the F⁻ counterparts were found to be similar to those of the F-merodiploid derivatives and the AO treatment did not result in selection of Rec⁺ UV^R revertants of the F⁻ strains.

The elimination of F30 by AO: This was measured as described in MATERIALS AND METHODS; the results are presented in detail in Figures 3 and 4, and for the purposes of comparison, some extracted data are presented in Table 3. No attempt was made to measure or calculate curing kinetics since segregation of multi-nucleate cells, reinfection by F30, and differences in growth rates of F30 and F⁻ cells may all contribute to the percent curing found after 20 hours.

The heterogeneity of the curing of strains derived from AB1157 (0-95%) is very different from the homogeneity of response of strains derived from JC411, all of which could be cured with a high efficiency (92-100%) by 25 µg/ml AO, except the strain hypersensitive to AO, JC4101.

FIGURE 3.—The curing of the F30 merodiploid derivatives of strains derived from JC411. The method used is described in the text.

In neither case was there any correlation between the Rec^- phenotypic group and the extent of curing.

It was surmised that in the two incurable strains, JC5415 and JC5416, the F30 might exist integrated into the chromosome, making it resistant to curing. However, the His^+ prototrophic conjugants formed by crossing these strains with JC2444 in liquid medium were all males carrying F30, since they gave $\text{His}^+[\text{Sm}^R]$ conjugants at high frequency with AB2463. Presumably, then, F30 is transferred, and so exists, as the autonomous F-merogenote.

The initial increase and then decrease in the efficiency of curing as the concentration of AO was increased suggests that the two main considerations for a

FIGURE 4.—The curing of the F30 merodiploid derivatives of strains derived from AB1157. The method used is described in the text.

high level of curing are that a high enough concentration of AO must be used, but that cell growth must not be completely inhibited. The increased permeability of *E. coli* to acridine dyes at alkaline pH's may explain why pH 7.8 is necessary for curing (SILVER, LEVINE, and SPIELMAN 1967). For both Rec⁺ and Rec⁻ strains, the smallest concentration of AO giving maximal curing was 25 µg/ml, and at higher concentrations, the curing of Rec⁻ strains decreased much more rapidly than that of Rec⁺ strains, presumably because of the greater growth inhibition. Inability to cure the strain hypersensitive to AO, JC4101, might be explained by complete growth inhibition at concentrations of AO too low to give any curing. The hypersensitivity itself may be due to a mutation separate from *rec-62*.

These experiments were extended to show that *Flac*⁺ derivatives of JC411, JC1553, AB1157, AB2470, and JC2690 could also be cured by 25 µg/ml AO, and thus that curing of Rec⁻ F-merodiploids is not a characteristic peculiar to F30.

Acridflavin uptake: It seemed possible that the difference in sensitivity towards AO of Rec⁺ and Rec⁻ strains might be due to differing abilities to take up the dye. Therefore the uptake of the related dye acridflavin by the strains used was measured by the method of SILVER and WENDT (1967), except that the uptake was measured using cells grown in L broth pH 7.80–7.90 to 4×10^8 /ml. AO itself could not be used as its fluorescence was too weak for it to be measured by this method. This was unfortunate since curing by acridflavin of either of the Rec⁺ strains was very inefficient (1–2%), and the relationship between acridflavin uptake and AO sensitivity and curing is therefore questionable. In any case, no consistent correlation was observed. In general Rec⁺ strains took up more acridflavin than their Rec⁻ counterparts, and F30 derivatives more than their F⁻ counterparts.

The incurability of AB2462 and AB2463 derivatives: These experiments were conducted to try to decide whether this incurability was a property due to the particular *rec* mutations involved, or to the presence of a second mutation in these strains.

Firstly, the *rec* alleles of AB1157, AB2462, and AB2463 were cotransduced with *cysC* into the Lac-Cys⁻ strain AT1378. *Flac*⁺ derivatives were made by crossing with E3, numbered JC5492, JC5493, and JC5494, respectively, and the curability of these was determined using various concentrations of AO. As shown in Figure 5, the Rec⁻ derivatives were cured to roughly the same extent as the Rec⁺ derivative; differences may be ascribed to the differing effects of AO on the growth of these strains.

Secondly, *Flac*⁺ derivatives were made of an UV^R Rec⁺ revertant of AB2462 and of an UV^R Rec⁺ transductant of AB2463 (constructed using P1 grown on an UV^R Rec⁺ strain), being JC5498 and JC5499, respectively. The slight increase in curability of JC5498 over JC5415 can probably be ascribed to its much improved ability to grow at a high concentration of AO. Although JC5499 segregates Lac⁻ derivatives at a high frequency, no increase was observed with up to 50 µg/ml AO.

Both of these types of experiment indicate that the inability of AO to cure F

FIGURE 5.—The curing of *Flac* merodiploid derivatives of *rec-cysC* cotransductants and of *Rec*⁺ revertants derived from AB2462 and AB2463. The method used is described in the text. Inhibition ratios were less than five except on the treatment of JC5493 and JC5494 with 25 µg/ml AO, when the inhibition ratios were both about 10⁶.

merodiploid derivatives of AB2462 and AB2463 is due to the presence of a second mutation in these strains distinct and separable from the *rec* mutation.

Variant strains: It was generally found that His⁺ survivors of the AO treatment could give rise to His⁺ prototrophic conjugants on crossing with the His⁻ female strain JC2444, and that His⁻ survivors could not. However, clones were occasionally found which did not conform to these characteristics, being either His⁺ non-donors or His⁻ donors (Table 4).

With the exception of the two clones derived from JC5417 and JC5415, His⁺ non-donor clones were all resistant to the male specific phage MS2 and did not segregate His⁻ progeny, either on growth in L broth or on treatment with AO. They were therefore classified as F⁻ His⁺ haploid recombinants.

TABLE 4
The variant strains formed on AO treatment

Parental strain	Phenotypic Rec group	Number* found	Aux ₁ [†]	His	Tfr ₁ [†]	Rtr ₁ [†]	MS2	Percent segregation of [‡]		Percent curing of [‡]	
								Tfr ⁺	His ⁺	Tfr ⁺	His ⁺
JC4087	+	6	+	+	—	..	R	..	0	..	0
JC4087	+	1	+	—	+	+	S	0	..	32	..
JC5414	+	5	+	—	+	+	S	0-2	..	79-88	..
JC5414	+	1	+	+	—	..	R	..	0	..	0
JC5417	1	1	+	+	—	..	R	..	1	..	62
JC5420	1	1	+	+	—	..	R	..	0	..	0
JC4101	3	1	+	+	—	..	R	..	0	..	0
JC5415	3	6	+	+	—	..	R	..	0	..	..
JC5415	3	1	+	+	—	..	S	..	0	..	..
JC5415	3	12	—	?	+	+	S	0-3	..	..	..

* About 600 single colonies from each strain were examined after treatment with various concentrations of AO.

[†] Aux is used to symbolize an additional unidentified auxotrophic requirement. Tfr⁺ (short for transfer) symbolizes the ability to sire His⁺ prototrophic conjugants with JC2444, and Rtr⁺ (short for retransfer) the ability of these conjugants to sire His⁺[Sm^R] conjugants on mating with AB2463.

[‡] Spontaneous segregation was measured after overnight growth in L broth, and curing by treatment with the concentration of AO previously found optimal for the parental strain. One hundred colonies were tested in each case.

Although the His⁺ non-donor clone derived from JC5417 was resistant to MS2, it segregated His⁻ progeny at a low frequency on growth in L broth, and at a high frequency on AO treatment. These His⁻ cells could be reinfected by F30 from JC4046 to give His⁺[Sm^R] conjugants, and so are presumably similar to the original F⁻ strain AB2470. This clone may carry a stable but non-transferring F30 derived by a mutation in F. Such mutations giving rise simultaneously to loss of transferring ability and MS2 resistance have been described previously (CUZIN 1962).

The His⁻ donor clones derived from JC5414 and JC4087 were found by similar methods to be F merodiploids in which the episome and chromosome carried the same known *his* alleles as in the parental strain, and no simple explanation of their formation could be deduced. However, gene conversion (MESELSON 1965) did not seem to have been involved.

JC5415 was unusual in giving a large proportion of variant strains of the type which were His⁺ but unable to give rise to His⁺ prototrophic conjugants with JC2444. Most of them were resistant to MS2 and were, therefore, either F⁻ haploid His⁺ recombinants or F mutants of the non-transferring MS2 resistant type. The latter possibility could not be ruled out in this case because of the difficulty of curing F-merodiploids derived from AB2462. Further study is necessary to characterize the clone which was still sensitive to MS2.

JC5415 also gave rise to a large number of clones with an unknown additional auxotrophic requirement. These mutations reverted at a frequency varying from 5×10^{-4} to less than 10^{-7} . They could give rise to His⁺ prototrophic conjugants

with the recipient JC2444, and these conjugants in turn could form $\text{His}^+[\text{Sm}^R]$ conjugants on crossing with AB2463. This showed that autonomous F30 was still present in these clones, ruling out the possibility that it had integrated into the chromosome to give rise to Hfr strains with an additional requirement by splitting of the genes at the sites of integration. In fact, strains with additional auxotrophic requirements could also be made by treatment of the F^- parent. AB2462, with AO, making the involvement of F30 unlikely. A generally high mutation rate may obtain in AB2462, perhaps leading to the high frequency of variant clones found, since the reversion rates of certain of the auxotrophic mutations carried are very high (A. J. CLARK, personal communication).

Most of the variant strains found arose from the Rec^+ merodiploids, which might be expected since recombination would be required for the formation of the His^+ non-donors, and possibly also for the His^- donors. Recombination in Rec_1^- strains can occur (B. LOW; see CLARK 1967), though at a reduced frequency, and would explain the F-His^+ haploid recombinant isolated from JC5420. However, the F-His^+ recombinant derived from the Rec_2^- strain JC4101 was unexpected. Although most of the variant strains were obtained after AO treatment of the F merodiploids, the data were not sufficient to determine whether AO was involved in their formation, and the experiments were not designed to identify all possible recombinant or mutant classes.

DISCUSSION

Our results confirm and extend previous reports (HIROTA 1960; STOUTHAMER, DE HAAN, and BULTEN 1963), which have shown that curing by acridines only takes place in actively dividing cells. Moreover, they clearly establish that a functional system for recombination is not essential for curing by AO, and therefore rule out recent suggestions that AO promotes curing only under conditions where recombination can occur (CLOWES and MOODY 1967). The two Rec^- strains showing normal AO sensitivity which when converted to F merodiploids cannot be cured by AO seem to be exceptional. CLOWES and MOODY (1967) have previously shown that one of these, AB2463, cannot be cured of the sex factor F either. The inability of AO to cure F merodiploids derived from these two strains is probably due to a second mutation distinct from the *rec* mutation. AO still inhibits the transfer of F30 from these strains to the same extent as from JC5414 at pH 7.8, however (WILLETTS, unpublished experiments). Further investigation of such mutants should increase our understanding of the mechanism of curing.

We therefore favor the original hypothesis of HIROTA (1960), JACOB, BRENNER, and CUZIN (1963), and STOUTHAMER, DE HAAN, and BULTEN (1963) of preferential inhibition of F replication to explain the majority of the curing by AO. As suggested by SILVER, LEVINE, and SPIELMAN (1967), some sort of compartmentalization may be involved, allowing access of the AO to the F replication system but not to the chromosomal replication system, since there should be no difference in AO binding to episomal or chromosomal DNA. At the concentra-

tion of AO which gave optimal curing, about 25 $\mu\text{g/ml}$, chromosomal replication was unaffected, at least in Rec^+ cells, as demonstrated by the unchanged growth. Inhibition of F replication is consistent with the AO inhibition of Hfr chromosomal transfer and of F-merogenote transfer found by JACOB, BRENNER and CUZIN (1963) and CUZIN and JACOB (1966), (though not by STOUTHAMER, DE HAAN, and BULTEN 1963), since such transfer may involve F-dependent replication (JACOB, BRENNER, and CUZIN 1963). However, these authors found an inhibition of F-merogenote transfer of 98%, whereas the kinetics of curing taken as such (STOUTHAMER, DE HAAN, and BULTEN 1963; WILLETTS 1967) would lead one to expect a lower inhibition of replication (25–50%). In a T6-interrupted mating between JC4087 and AB1157 at pH 7.8 (WILLETTS, unpublished experiments), F30 transfer was inhibited by only 75%. This, together with the possibility of some reinfection of cured cells during the AO treatment, is more in accord with the curing kinetics.

In other systems, the mechanism of curing may be different. For example, curing by chemical mutagens and UV (WILLETTS 1967), and by ICR derivatives (CLOWES and MOODY 1967), may involve mutagenesis and in the strain used by CURTISS (1968), which carried a linear chromosomal merogenote, recombination may be essential for elimination of the merogenote: this occurred at a much lower efficiency than AO curing of an F-merogenote, and AO had no effect in this system (CURTISS 1964). In the F-merogenote system used in the present studies, elimination by recombination may occur as a small proportion of the total elimination promoted by AO in Rec^+ strains, perhaps giving rise to the several His^+ F⁻ clones which were found.

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SUMMARY

Both Rec^- F⁻ strains and their F30 derivatives were more sensitive to acridine orange than the Rec^+ ancestors of their F30 derivatives, but there was no consistent correlation with UV sensitivity. Nine out of twelve F30 derivatives of Rec^- strains representative of each of CLARK's three phenotypic groups could be cured with high efficiency by acridine orange: the incurability of two of the remaining strains was shown to be due to a second mutation separable from the *rec* mutation. Thus an operational system for recombination is not essential for curing. Recombinant and mutant clones were isolated after the acridine orange treatment, and these were partially characterized.

The reference CLOWES and MOODY (1967) refers to a personal communication.

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