ACRIDINE-RESISTANT MUTANTS OF T2H BACTERIOPHAGE

ANITA Y. HESSLER[1]

Marine Biological Laboratory, Woods Hole, Massachusetts

Received March 14, 1963

THE mechanisms which prevent formation of infective T2H bacteriophage in *Escherichia coli* in the presence of acridine dyes are unknown. That interruption of normal maturation occurs in a late stage of phage development was suggested by the work of FOSTER (1948) and has been substantiated physiologically and cytologically. DEMARS, LURIA, FISHER and LEVINTHAL (1953) and DEMARS (1955) found in proflavine lysates accumulations of near normal amounts of DNA, serum blocking power, and empty heads ("doughnuts"); KELLENBERGER and SECHAUD (1957) found empty heads and tail-like rods but few intact phage in their electron microscopical studies of proflavine lysates. Acridines also may interfere in the actual completion of DNA synthesis. The electrophoretic studies of ASTRACHAN and VOLKIN (1957) showed structural differences between T2 DNA extracted from a proflavine lysate and that from a normal lysate. LERMAN (1961, 1963), by means of sedimentation and viscosity measurements and X-ray diffraction studies, found support for the idea that proflavine or quinacrine molecules which were bound to DNA in a phage-dye complex alter the structure of the DNA molecule by intercalation between base pairs. The well-known mutagenic properties of acridines (DEMARS 1953) have been attributed by ORGEL and BRENNER (1961) to changes in the structure of the DNA molecule, possibly the deletion or addition of bases (BRENNER, BARNETT, CRICK, and ORGEL 1961; CRICK, BARNETT, BRENNER, and WATTS-TOBIN 1961).

A study of acridine-resistant mutants of T2, i.e. phage which can grow in proflavine or other acridine dyes, might prove useful in elucidating the sequence of maturation steps or in contributing to understanding of resistance of the infected cells. Four acridine-resistant mutant loci have been mapped genetically and studied to a limited extent physiologically to learn something of their characteristic action. Although the array of places where acridines could act during development complicates the study of resistance to dye action, the present study permits the conclusion that the four mutants represent two unlinked genetic loci which have separate effects on phage maturation. SUSMAN (1963, personal communication), in an earlier study of the action of acridines on T4 bacteriophage development, found two acridine-resistant loci, designated as *ac* and *q* (for location on T4 linkage map see PRATT, STENT and HARRIMAN 1961). The T2H mutants resemble those of T4 both in location and behavior.

[1] This investigation was supported by Research Grant AI-02173 from the National Institutes of Health, Public Health Service, to DR. MARTHA B. BAYLOR and the author.

MATERIALS AND METHODS

Bacterial strains: The host cell for crosses or single-step growth experiments was *E. coli* B obtained from DR. G. STREISINGER. Plating cells were S and 2bc (B/2H), and occasionally K-12λ.

Virus stocks: Stocks of dye-resistant mutants were made either as lysates in synthetic medium or as S-cell agar plate lysates. Besides the four resistant mutants, stocks of T2 wild type, *h* (host range) mutant, various *r* (plaque morphology) mutants, and *ht* (intermediate host range) mutants (BAYLOR, HURST, ALLEN and BERTANI 1957) were used for mapping purposes (Figure 1; Table 1).

Media: Nutrient broth was composed of Bacto-peptone, 6 g; Bacto beef extract, 1.8 g; NaCl, 3.0 g; glucose, 0.6 g; H_2O, 1 liter. M9 synthetic medium, bottom layer agar, and soft plating agar were prepared as described by BAYLOR *et al.* (1957).

Acridine dye stock solutions: The name, commercial source, and stock solution concentration of the three acridine dyes used are these: Proflavine (3,6-diamino-

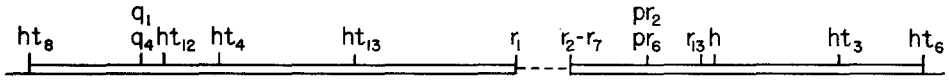

FIGURE 1.—Partial linkage map of T2H. The acridine-resistant mutants q_1 and q_4, pr_2 and pr_6 are located among known markers diagrammatically (see Table 1 for details of three-factor crosses). Total length between ht_8 and r_1 is 90 recombination units, the sum of the percent recombination between adjacent markers; between the r_2-r_7 region and ht_6 is a total of 65 recombination units. The r_1 and r_2-r_7 regions have been linked in experiments of STREISINGER and BRUCE (1960).

TABLE 1

Location of pr *and* q *mutants on the linkage map of T2H. Relative map distance in terms of percentage recombination between pairs of markers in three-factor crosses*

Parental genotypes	Number of plaques				Correction factor*
		r-h	*r-q*$_1$	*q*$_1$*-h*	
$r_{13} \times h\, q_1$	146	3.4	37.5	36.1	1.25
$r_2 h \times q_1$	616	20.7	30.2	39.5	1.30
		r$_1$*-ht*	*r*$_1$*-q*$_1$	*ht-q*$_1$	
$r_1 \times ht_{13} q_1$	1216	31.7	36.4	20.1	1.20
$r_1 q_1 \times ht_{13}$	559	26.6	29.8	19.7	1.25
$r_1 ht_{12} \times q_1$	212	28.0	31.5	7.1	1.50
$r_1 ht_8 \times q_1$	250	45.0	47.0	21.0	1.50
		ht$_{13}$*-ht*$_4$	*ht*$_4$*-q*$_1$	*ht*$_{13}$*-q*$_1$	
$ht_{13} q_1 \times ht_4$	800	11.0	13.7	22.2	1.05
		r$_1$*-h*	*r*$_1$*-q*$_4$	*q*$_4$*-h*	
$r_1 \times h q_4$	594	37.5	35.8	31.0	1.53
		r-h	*r-pr*	*pr-h*	
$r_{13} pr_2 \times h$	1391	1.7	10.6	11.1	1.16
$r_{13} h \times pr_6$	718	3.6	14.2	14.6	1.29
$r_7 \times h\, pr_6$	967	...	13.7	12.0	1.20
	1642	18.8	...	...	1.20

* LENNOX, LEVINTHAL, and SMITH (1953).

acridinium diHCl, Mann Research Laboratories, Inc.), 200 μg/ml; Acriflavine Neutral IX (Nutritional Biochemicals Corporation), 100 μg/ml; and Quinacrine HCl (Mann Research Laboratories, Inc.), 10 mg/ml. All dyes were prepared separately in 100-ml lots of sterile distilled water, kept refrigerated in the dark, and discarded every two weeks to insure freshness.

Nomenclature: The symbols *pr* and *q* were adopted for the new mutants after extensive genetic mapping and single-cycle growth experiments in dye-supplemented media revealed two unlinked genetic sites which conferred resistance primarily either to proflavine or quinacrine.

Crosses: Crosses were performed in the standard manner, as outlined by ADAMS (1959). Freshly grown B cells which were washed in buffer were infected with six to eight phage per cell (parental ratio from 0.6 to 1) at 37°C for ten minutes before 1:100 dilution into broth. Growth of phage for 15 minutes was then permitted before premature lysis of growth-tube contents took place with the addition of chloroform. Adsorption tubes were assayed for free phage and residual bacteria to arrive at an estimate of total phage input. After lysis, the contents of growth tubes were diluted and plated on appropriate indicator cells. The replica plating technique of LEDERBERG and LEDERBERG (1952) was used as modified by BAYLOR *et al.* (1957) to distinguish phenotypes. Preliminary experiments with proflavine-supplemented agar plates showed that the resistant mutants *pr* (proflavine and acriflavine resistant), *q* (quinacrine resistant), and *pr q* (double mutant recombinant obtained in *pr* × *q* cross) can transfer well to S cells grown on a concentration of 4 μg/ml of proflavine, whereas T2 wild phage cannot. The direct plating of the progeny of crosses to proflavine-supplemented agar does not invariably distinguish resistant from sensitive phage and was thus not employed as a screening technique in crosses. Velvet transfer of master plates from crosses involving *ht* markers to replica plates containing 2bc (B/2H) cells distinguished the *ht* from ht^+ phenotypes; similar transfer to K-12λ from S cells clearly distinguished the *r* and r^+ forms in crosses involving r_2 or r_7.

Single step growth experiments: The single-step growth experiment was used extensively to compare growth patterns of mutant and wild-type infected complexes under normal conditions and in dye. Infections were either monoparental, involving one kind of parental phage, but often with a multiplicity of five or more phage per cell, or biparental, that is, mixed infections. Observations of numbers of phage present at a given time were made by assay at intervals throughout the growth period or at the end of the lytic period. Assay of the adsorption tube for free phage showed that these amounted to less than 0.1 percent of the initial infective particles in each growth tube. Bacteria were infected and diluted after adsorption into broth to a concentration of 10^4 cells/ml; one half of this control growth tube was then diluted into broth containing twice the desired end concentration of dye to insure a common source of the infected cells in control and experimental tubes. At the end of the growth period, a few drops of chloroform were added to each growth tube Then the growth tubes were assayed to compare the final yield from the lysate in dye with the yield in untreated broth.

General note: Precautions were taken to guard all dye-supplemented media and experimental growth tubes from exposure to light since it is known that phage-proflavine complexes are photosensitive. HIATT (1960) showed that free bacteriophage T2r^+ previously incubated in 4 μg/ml of proflavine is inactivated when exposed to visible light. Preliminary experiments in this laboratory suggest that infective centers in the presence of dye are very sensitive to light.

RESULTS

Genetic mapping: The new mutants were obtained initially by plating T2 Hershey wild-type stocks on agar supplemented with proflavine (5 μg/ml) and picking and purifying the occasional resistant phage plaque which appeared there. To locate the new *pr* and *q* mutants, three-factor crosses were performed which provided an internal control cross of two known markers (*r* and *h* or *ht*). A summary of the results (Figure 1; Table 1) confirms the relative order and map distances which BAYLOR *et al.* (1957) established for the known markers. The new mutants are unambiguously located within this framework.

To establish whether the pairs of mutants were allelic, pr_6 and pr_2 were crossed, as were q_1 and q_4. Also *pr* and *q* were crossed with one another in various combinations. The results of these crosses (Table 2) confirmed the total lack of linkage between *pr* and *q*, showed 1.4 percent recombination between q_1 and q_4 and less than 0.05 percent recombination between pr_2 and pr_6.

All three-factor crosses were corrected for monoparental infections and for differences in parental input ratio according to LENNOX, LEVINTHAL, and SMITH (1953). The extent of correction in each cross is indicated in Table 1. The data for two-factor crosses are uncorrected.

Single-step growth experiments: Single cycles of growth were studied in both untreated and acridine-supplemented infected complexes to determine characteristic patterns of development for resistant and sensitive phages. KELLENBERGER and SECHAUD (1957) reported for T4 a lag in first appearance of infectious phage grown in various concentrations of proflavine and very low final productivity

TABLE 2

Percentage recombination between acridine-resistant mutants pr *and* q *(expressed as twice the number of wild-type recombinants)*

Parental genotypes		Number of plaques per cross	*pr-q*	*pr-pr* or *q-q*	Average percent recombination*
$pr_6 \times q_1$	(1)	340	39.6	. .	. . . . .
	(2)	400	36.6	. .	. . . . .
	(3)	168	31.0	. .	. . . . .
	(4)	350	51.0	. .	39.5 ± 7.7
$pr_2 \times q_1$	(1)	1034	38.4	. .	. . . . .
	(2)	375	53.0	. .	45.7 ± 7.3
$pr_2 \times pr_6$		2209	. . .	0	< 0.05
$q_1 \times q_4$		1248	. . .	1.4	1.4

* Data from the crosses are uncorrected. ± the standard error of the mean.

of single infected cells, phenomena which have been investigated in detail by SUSMAN and RITCHIE (1963). Our studies with T2H include observations of the effects of quinacrine and acriflavine as well as proflavine. The phenotypes of the dye-resistant mutants and of sensitive phages were established in terms of their characteristic differences in capacity to grow in the presence of each dye. Tolerance of both resistant and sensitive infected complexes decreases as dye concentrations are raised. Resistance is not an all or none phenomenon.

1. *Proflavine:* T2-infected complexes grown in proflavine show different effects depending on the quantity of dye present during growth. Thus 4 μg/ml or more causes death of infective centers, whereas 2 μg/ml permits a minimal production of new phage, and lower concentrations are less inhibitory. The action of this acridine is not simply one of indefinite lysis inhibition. When aliquots of infected cells are diluted out of dye at intervals throughout the normal lytic phase and permitted to develop in untreated broth, the number of phage produced in these dilute growth tubes is progressively smaller, the longer the cells have been in dye. A qualitative difference was noted when growth tubes containing either 4 μg/ml or 2 μg/ml proflavine were each diluted into plain warm broth prior to normal time of lysis. (The time of dilution was 16 minutes after the beginning of growth in broth after a ten-minute adsorption period or a total of 26 minutes after the initial infection was made.) A time lag of about eight minutes beyond the onset of lysis in untreated samples preceded release of mature phage after infected complexes had been exposed to 4 μg/ml proflavine (Figure 3). No such lag is observed when the complexes have been held in 2 μg/ml proflavine for a comparable period prior to dilution into broth.

The growth pattern of an infection made with *pr* differs from that of T2. The number of phage recovered from cells infected with *pr* in 4 μg/ml of proflavine was lower than in 2 μg/ml, but release of mature phage occurred at the same time as in untreated infections. The resistant mutant *pr* lysed in 2 μg/ml proflavine within four minutes after the untreated control complexes and rapidly produced a maximum yield in dye, often a normal yield, at the same rate as did untreated infected complexes (Figure 4). Cells infected with *pr*, if diluted out of dye and into broth prior to lysis as explained above, gave a full complement of phage at the same time as did the untreated sample. In 8 μg/ml proflavine, *pr* does not grow at all.

The double mutant *pr q* gave a full yield of infective phage on time in both 2 μg/ml and 4 μg/ml of proflavine. In 8 μg/ml proflavine, the double mutant gave from 10 to 30 percent of a normal yield. SUSMAN (personal communication) found that *ac q*-infected complexes of T4 are more resistant to proflavine than either *q*-, *ac*-, or T4-infected complexes, in that order.

2. *Quinacrine:* When similar experiments were carried out using quinacrine in place of proflavine, and a comparison is made between quinacrine-sensitive and resistant phage, essentially the same results were obtained. T2 infected complexes in the completely inhibitory concentration of 100 μg/ml quinacrine gave no phage, and when diluted into broth prior to normal time of lysis, they showed a distinct 15-minute lag before new phage began to be recovered (Figure 3). In 50

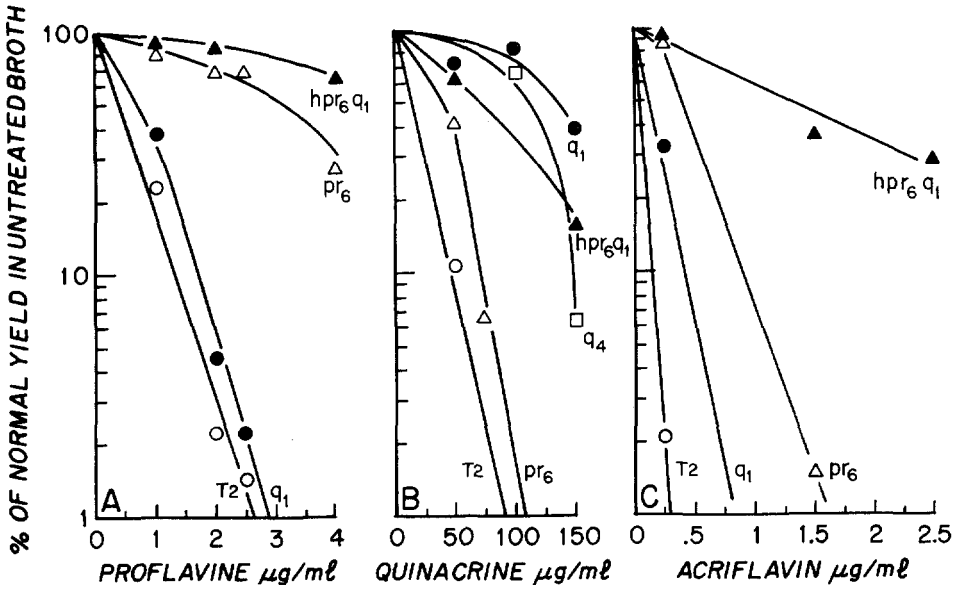

FIGURE 2.—Comparative yields of phage by *E. coli* cells infected in the presence of various acridine dyes. Multiplicity of infection: two to five phage per bacterium. Free phage amounted to no more than 0.1 percent of the initial assay of infective particles. The quantities of dye indicated are the amount present during adsorption (10 minutes) and in broth growth tubes (35 minutes) at 37°C in the dark before assay. The yield in dye is given as the percent of a normal yield in an untreated broth growth tube which accompanied each infection in dye. Open circles = T2H. Solid circles = q_1. Open squares = q_4. Open triangles = pr_6. Solid triangles = $h\ pr_6q_1$.

or 75 μg/ml quinacrine, less inhibitory concentrations, T2 infected cells yielded reduced numbers (about ten percent of a normal sized yield) at the same time an untreated infection gave its normal yield. When diluted out of these low quinacrine concentrations before normal lysis time, T2 infected cells gave a full yield at the same time as an untreated infection.

The *q* mutant, if grown in 50, 75, or 100 μg/ml of quinacrine, yielded from 100 to 70 percent of a normal burst at the same time as lysis occurred in an unsupplemented growth tube. However, when the *pr* mutants were grown in a series of quinacrine concentrations, they yielded a few phage in 50 μg/ml and showed no new phage at all if grown in 100 or 150 μg/ml of quinacrine. A double mutant infection with *pr q* did yield new phage in 150 μg/ml of quinacrine, but less than did the *q* mutant grown alone. The infected complexes of *pr q* were significantly more resistant to quinacrine than were those of the single mutant *pr*. (See Figure 2, B.)

3. *Acriflavine:* When T2 was grown in .75 μg/ml of acriflavine, a decrease of infective centers occurred. If grown in .25 μg/ml of this dye, a small number of phage (two per input bacterium) were produced. The *q* mutant, while completely sensitive to .75 μg/ml acriflavine, generally gave about 20 percent of a normal yield at .25 μg/ml and thus is relatively less sensitive to acriflavine during growth than is T2.

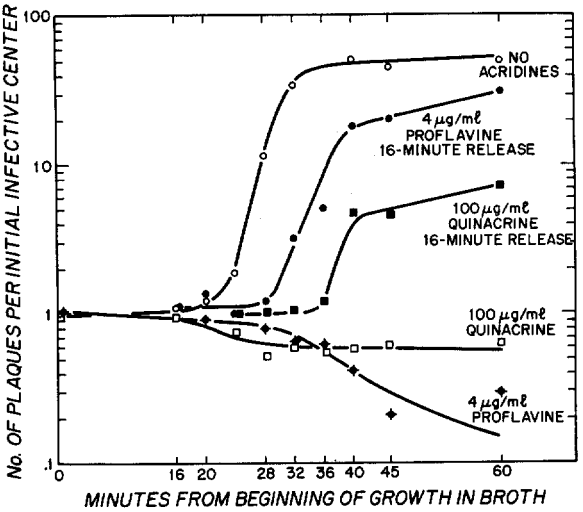

FIGURE 3.—Comparative growth patterns of T2 in proflavine and quinacrine. A sample of each supplemented growth tube was released from dye by dilution 1:100 at 16 minutes after the commencement of growth in broth. Multiplicity of infection was five phage per bacterium; free phage amounted to no more than 0.1 percent of the initial assay of infective particles. Adsorption period was ten minutes at 37°C before dilution into broth growth tubes. All growth tubes were kept in the dark throughout incubation. Open circles = T2, no dye; solid circles = T2 released from 4 μg/ml proflavine prior to lysis. Solid crossed circles = 4 μg/ml proflavine present throughout 60-minute growth period. Solid squares = T2 released from 100 μg/ml quinacrine at 16 minutes, prior to lysis; and open squares = 100 μg/ml quinacrine present throughout 60 minutes.

Infections made with the *pr* mutants or the double mutant *pr q* yielded full complements of phage in .25 μg/ml acriflavine at the normal time of lysis for an untreated infection. At high acriflavine concentrations, such as 1.5 μg/ml and 2.5 μg/ml, the *pr q* infected complexes were found to yield progeny, whereas the single *pr* mutant infected complexes did not. The *ac q* mutant of T4 grows well on 2.75 μg/ml of acriflavine (PRATT, *et al.* 1961), whereas *ac*, *q*, and wild type in that order, are more sensitive to acriflavine (SUSMAN, personal communication).

The relative differences in efficiency of growth in the various acridines outlined above account for the main distinction between the *pr* and *q* loci (see Figures 2A, B, C). Cells infected with a *pr* mutant yield infective particles in the presence of proflavine or acriflavine at concentrations in which *q* or q^+ infections do not yield phage. Conversely, infected complexes of *pr* mutants yield few phage in concentrations of quinacrine in which the *q* mutants grow. (The q_4 infected complexes showed lower tolerance for high quinacrine concentrations than do q_1 infected complexes.) Cells infected with T2 fail to yield phage in all three dyes at concentrations tolerable to the appropriate resistant single mutants. A double *pr q* mutant can grow in proflavine or acriflavine supplemented medium at higher dye concentrations than can either single mutant, and so the double mutant is said to be additive in effect. The growth of *pr q* in the presence of quinacrine, though superior to *pr*, is generally about equal to that of *q*-infected complexes.

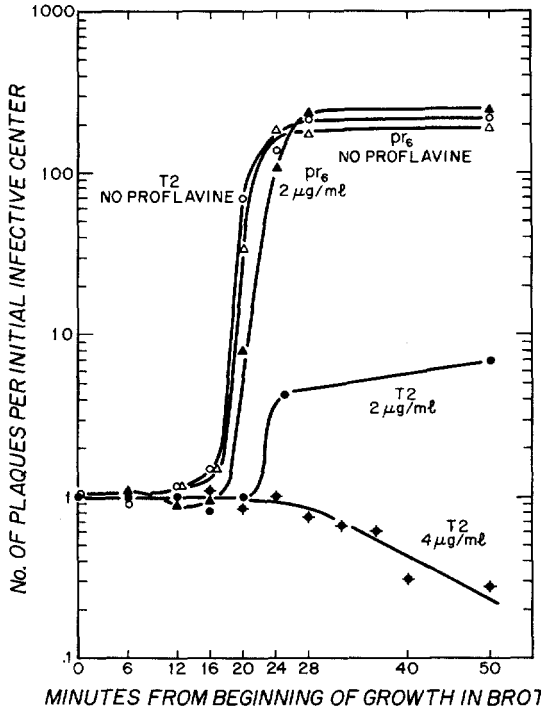

FIGURE 4.—Comparison of growth of T2 and pr_6 in single-step growth experiments in broth supplemented with proflavine, or not supplemented. Multiplicity of infection: five phage per bacterium. Free phage amounted to no more than 0.1 percent of the initial assay of infective particles. Samples withdrawn at intervals were not chloroformed; terminal sample was chloroformed. Adsorption and growth tubes incubated at 37°C in the dark. Open circles = T2, no dye; solid circles = T2, 2 μg/ml proflavine; solid crossed circles = T2, 4 μg/ml proflavine. Open triangles = pr_6, no dye; solid triangles = pr_6, 2 μg/ml proflavine.

Finally, mutant *pr* or *q* stocks show characteristic tolerances to a given dye during growth which are consistent regardless of their genetic composition with respect to other mutant loci not concerned primarily with dye resistance or sensitivity.

The additive nature of the double mutant *pr q* in infected cells grown in the presence of either proflavine or acriflavine is expressed in the fact that far more progeny can mature than when the *pr* or *q* mutant is the sole kind of infecting phage (Figures 2, A, C). This suggests the possibility that coordination of gene products occurs in the case of the *pr q* infection. Conversely, the sensitivity of the q^+ function in *pr*-infected cells and some effect of the pr^+ function in *q*-infected cells may yield suboptimal conditions for phage maturation of the two single mutant functions *pr* or *q* in proflavine or acriflavine. The results of crosses which bear on this point will be discussed below.

The *pr q* mutant grown in quinacrine is not more efficient, and in some concentrations is less efficient, in yielding phage than is the *q* mutant grown alone. Both infections made with *pr q* and *q*, however, are significantly more productive

than *pr* infected complexes in this dye. It is conceivable that sensitivity of the q^+ function present in its genome affects the development of *pr* mutants in quinacrine.

Although proflavine, acriflavine, and quinacrine block production of infectious phage in T2-infected cells, by no means does this imply identical actions for the dyes. Experiments were performed wherein a *pr* mutant was grown in a mixture of 2.0 μg/ml of proflavine plus 50.0 μg/ml of quinacrine. At the same time a *q* mutant was grown separately in an identical mixture; the *pr q* double mutant was also grown separately in the same mixture of dyes. This experiment was repeated using dye concentrations of 2.5 μg/ml of proflavine and 75.0 μg/ml of quinacrine, respectively. The concentrations in these experiments were selected so as to expose each single mutant to a tolerable concentration of at least one of the dyes in the mixture. In the subinhibitory amounts of proflavine alone there was no difference in the yields of *pr* and *pr q*. Also the intermediate concentrations of quinacrine resulted in wide variation within any one experiment, and in both cases presented, *q* grew far better than did *pr q*. Nevertheless, when either a *pr* or *q* mutant was grown in the mixtures of proflavine plus quinacrine, only a few phage per bacterium were recovered (see Table 3). In contrast, when the double mutant *pr q* was grown in each dye mixture, a significantly greater yield of phage was obtained than from the single mutant infections. This suggests that the response of the *pr* mutants to proflavine consists in correcting a block to maturation which differs from the block which *q* mutants can correct in the presence of quinacrine. The presence of both resistant loci and the absence of the sensitive ones in *pr q* infected complexes growing in both dyes apparently combats the action of each acridine. It is probable that this counteraction is either sequential (with the action of one mutant locus preceding that of the other) or is concerned with two separate simultaneous patterns of acridine action. Plainly it would be of great interest to determine which case is true.

Crosses in the presence of dye: A series of crosses between dye-resistant and dye-

TABLE 3

Growth of acridine-resistant mutants in broth supplemented with either one or two acridines. Values are percent of the yield in unsupplemented broth

Acridine	Concentration μg/ml	Genotype of mutant		
		pr_6	q_1	$h\ pr_6\ q_1$
Experiment 1				
Proflavine	2.0	80	2	80
Quinacrine	50.0	26	60	33
Proflavine and quinacrine	2.0 + 50.0	8	6	70
Experiment 2				
Proflavine	2.5	42	2	46
Quinacrine	75.0	1	82	31
Proflavine and quinacrine	2.5 + 75.0	3	1	23

See discussion of these experiments in RESULTS section.

sensitive phages were performed in the presence of various subinhibitory proflavine concentrations throughout a 35-minute growth cycle. These crosses were performed to examine both the productivity of the infected cells and the genetic constitution of the progeny.

1. *Phage production:* A summary of several crosses performed in proflavine-supplemented broth shows the following relation between yield of phage obtained and genotype of the input parental phage (Table 4). The progeny of $pr \times pr^+$ in dye are intermediate numbers of phage—more than the yield from a sensitive infection alone, fewer than arise from a resistant infection. The result of crossing either *pr* and *q* or *pr q* and $pr^+ q^+$ is a lower yield than a *pr q* infection gives alone, but it is more than from wild type infected complexes alone.

2. *Analysis of genotypes:* The distribution of genetic markers among the progeny from both untreated growth tubes and those exposed to proflavine was tested to determine whether the progeny recovered from mixed infections might have an atypical representation of phenotypes if dye was present during development. Despite considerably lower phage production than in untreated infected complexes, classes of all phenotypes were recovered from dye-supplemented growth tubes. The percentage recombination was not significantly different in dye and in untreated growth tubes within each cross.

For example, when three crosses were performed using $h\ q_1$, $h\ pr_6$, and $h\ pr_6\ q_1$, respectively, with r_1 in the presence of 2 μg/ml of dye, the total progeny recovered, compared to normal-sized yields of 100 percent from untreated crosses were: 13 percent for $h\ q_1 \times r_1$, 22 percent for $h\ pr_6 \times r_1$ and 40 percent for $h\ pr_6\ q_1 \times r_1$. In each case these numbers were intermediate between the recovery expected from the r_1 grown alone and each of the other parents grown alone. In all three crosses, recovery of all phage classes, both parental and recombinant for r_1, *h*, or dye-resistant markers, was recorded; but the recovery was simply of lower numbers of each class than in untreated growth tubes, and this then is the major difference between normal crosses and those performed in proflavine.

TABLE 4

Comparative phage yield in proflavine from single parent and mixed infections of E. coli

Genotype of input phage	Number of experiments	2.0 μg/ml proflavine*	Genotype of input phage	Number of experiments	2.5 μg/ml proflavine*
T2	10	2.2 ± 2.7	T2	3	1.4 ± 0.5
$r_1 q_1$	4	18.3 ± 15.1	$r_{13} pr_2$	1	53.0
T2 × $r_1 q_1$	1	8.9	T2 × $r_{13} pr_2$	3	17.0 ± 6.3
$r_1 \times h\ q_1$	1	13.0			
r_1	1	7.0	r_{13}	4	5.7 ± 3.4
$h\ pr_6 q_1$	5	84.7 ± 13.7	pr_6	5	67.5 ± 13.8
$r_1 \times h\ pr_6 q_1$	2	54.0 ± 14.0	$r_{13} \times pr_6$	3	27.9 ± 3.2
$r_{13} pr_2$	1	58.0	$h\ q_4$	2	3.7 ± 2.0
$r_1 q_1 \times pr_2$	2	44.0 ± 8.0	$r_{13} pr_2$	1	53.0
$q_4 \times r_{13} pr_2$	1	35.0	$r_{13} pr_2 \times h q_4$	2	20.5 ± 10.0

* Values are percent of the yield in unsupplemented broth, ± the standard error of the mean.

Although it is not clear what the mechanisms of interaction are between the mutant *pr* and *q* loci, it seems likely that the wild type opposite functions pr^+ and q^+ may be acting to affect the efficiency of phage production. This is evident from the growth experiments which compare efficiency of growth of the double mutant *pr q* with that of each single mutant in mixtures of acridines. The crosses in dye further indicate that efficiency of phage maturation is a resultant of the operation of both the resistant and sensitive functions associated with the parental input phage. It is of interest that T4 mixedly infected with *ac* and ac^+ are unproductive in proflavine and acriflavine concentrations which do not inhibit *ac* alone (SUSMAN, personal communication); thus ac^+ appears dominant to the *ac* function (EDGAR and EPSTEIN 1961).

DISCUSSION

In establishing the characteristic efficiencies of growth in acridine dyes displayed by acridine-resistant or acridine-sensitive phages, we have recognized several interesting problems. Precisely what processes proflavine, acriflavine, and quinacrine block and the manner in which the mutant functions *pr* and *q* act to circumvent these blocks is of principal importance. An interpretation of the action of acridines on phage growth and the counteraction by the resistant functions must explain both the nature of the additivity of double (*pr q*) mutants as well as the results of mixed infections of resistant and sensitive phages in proflavine.

The actions of proflavine and acriflavine upon phage-infected cells may be similar; that of quinacrine may differ radically from the other two dyes. The degree of resistance among the genotypes studied is, in general, $pr\,q > pr > q >$ T2 in infected complexes grown in proflavine or acriflavine, whereas $q = pr\,q >$ $pr >$ T2 in quinacrine. Bacteria infected with *pr q* phage produce significant yields in a mixture of either proflavine or acriflavine plus quinacrine which is sufficient to prevent either single mutant from maturing when grown alone. These observations strongly support the contention that the *pr* and *q* functions are related to different maturation steps. Support for this is found in the lag in development observed when T2-infected cells were released from inhibitory concentrations of either proflavine or quinacrine. The period of lag was consistently greater when the inhibition of quinacrine was released than was the case of release from proflavine.

The results of crosses suggest that the successful development of phage in subinhibitory concentrations of proflavine arises from interactions between the products of resistant and sensitive loci of the parental input phages. The complete and unselected array of parental and recombinant genotypes recovered from three-factor crosses in proflavine is evidence that the dye is not hindering that stage in DNA development when recombination occurs. That low yields of phage recovered from dye-supplemented growth conditions have essentially the same percentage distribution of genotypes as progeny of untreated infected complexes implies that a smaller sample of phage replicates is made into mature phage when proflavine is present. Thus the rate of formation of mature particles is slower in pro-

flavine than in normal growth conditions. To give this effect, some of the areas that proflavine could block are (1) the finishing of the DNA (such as proper coiling thereof), (2) synthesis of external proteins which compose phage coats, or (3) assembly of mature phage. Indeed it is possible that proflavine has a complex effect on development.

SUMMARY

Two classes of acridine-resistant functions, *pr* and *q*, of T2H bacteriophage of *Escherichia coli* are mapped genetically and described. In inhibitory concentrations of proflavine, quinacrine, or acriflavine T2 wild-type infected complexes cannot grow. Cells infected with *pr* can grow in proflavine or acriflavine; cells infected with *q* yield phage in quinacrine; cells infected with *pr q* yield phage in all three acridines. The double mutant *pr q* displays additivity in growing better in proflavine or acriflavine than either single mutant. The phage yield from crosses performed in proflavine is reduced to a point midway between that expected of the resistant or the sensitive phage parent when either parent is grown alone in the dye.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my colleague, Dr. Martha B. Baylor, for acquainting me with bacteriophage genetics, and for most encouraging and fruitful discussions on this and other research problems. I am also grateful to Drs. Simon Silver and D. A. Ritchie for their suggestions and criticism of the manuscript. The help of Mrs. Violet Gifford in performing certain of the experiments was most appreciated.

LITERATURE CITED

Adams, M. H., 1959 *Bacteriophages.* Interscience Publishers, Inc., New York.

Astrachan, L., and E. Volkin, 1957 Chromatographic detection of differences betwen bacteriophage-related deoxyribonucleic acids. J. Am. Chem. Soc. **79:** 130–134.

Baylor, M. B., D. D. Hurst, S. L. Allen, and E. T. Bertani, 1957 The frequency and distribution of loci affecting host range in the coliphage T2H. Genetics **42:** 104–120.

Brenner, S., L. Barnett, F. H. C. Crick, and A. Orgel, 1961 The theory of mutagenesis. J. Mol. Biol. **3:** 121–124.

Crick, F. H. C., L. Barnett, S. Brenner, and R. J. Watts-Tobin, 1961 General nature of the genetic code for proteins. Nature **192:** 1227–1232.

Demars, R. I., 1953 Chemical mutagenesis in bacteriophage T2. Nature **172:** 964.

1955 The production of phage-related materials when bacteriophage development is interrupted by proflavine. Virology **1:** 83–99.

Demars, R. I., S. Luria, H. Fisher, and C. Levinthal, 1953 The production of incomplete bacteriophage particles by the action of proflavine and the properties of the incomplete particles. Ann. Inst. Pasteur **84:** 113–128.

Edgar, R. S., and R. H. Epstein, 1961 Inactivation by ultraviolet light of an acriflavine-sensitive gene function in phage T4D. Science **134:** 327–328.

Foster, R. A. C., 1948 An analysis of the action of proflavine on bacteriophage growth. J. Bacteriol. **56:** 795–809.

HIATT, C. W., 1960 Photodynamic inactivation of viruses. Trans. N. Y. Acad. Sci. Ser. II, **23**: 66–78.

KELLENBERGER, E., and J. SECHAUD, 1957 Electron microscopical studies of phage multiplication. II. Production of phage-related structures during multiplication of phages T2 and T4. Virology **3**: 256–274.

LEDERBERG, J., and E. M. LEDERBERG, 1952 Replica plating and indirect selection of bacterial mutants. J. Bacteriol. **63**: 399–406.

LENNOX, E. S., C. LEVINTHAL, and F. SMITH, 1953 The effect of finite input in reducing recombinant frequency. Genetics **38**: 508–511.

LERMAN, L. S., 1961 Structural considerations in the interaction of DNA and acridines. J. Mol. Biol. **3**: 18–30.

1963 The structure of the DNA-acridine complex. Proc. Natl. Acad. Sci. U.S. **49**: 94–102.

ORGEL, A., and S. BRENNER, 1961 Mutagenesis of bacteriophage T4 by acridines. J. Mol. Biol. **3**: 762–768.

PRATT, D., G. S. STENT, and P. D. HARRIMAN, 1961 Stabilization of 32P decay and onset of DNA replication of T4 bacteriophage. J. Mol. Biol. **3**: 409–424.

STREISINGER, G., and V. BRUCE, 1960 Linkage of genetic markers in phages T2 and T4. Genetics **45**: 1289–1296.

SUSMAN, M., and D. A. RITCHIE, 1963 Manuscript in preparation.