ACRIDINE RESISTANCE IN BACTERIOPHAGE T2H AS A FUNCTION OF DYE PENETRATION MEASURED BY MUTAGENESIS AND PHOTOINACTIVATION

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PROFLAVINE interferes with the development of the *E. coli* bacteriophage T2, stopping the formation of infective particles, apparently without affecting production of DNA and protein components of mature phage (FOSTER 1948; DEMARS 1955; KELLENBERGER and SECHAUD 1957). The isolation of the acridine resistant mutants *pr* and *q* (HESSLER 1963) for T2 and *ac* and *q* (SUSMAN, referred to by PRATT, STENT, and HARRIMAN 1961) for T4 has provided access to the complex problem of how proflavine acts. These mutants confer resistance to proflavine on the infected complex and allow normal phage development in the presence of proflavine concentrations which inhibit wild type.

The mutagenic effects of acridines, especially proflavine, during phage development have been studied by DEMARS 1953; BRENNER, BENZER, and BARNETT 1958; ORGEL and BRENNER 1961; BRENNER, BARNETT, CRICK, and ORGEL 1961; and DRAKE 1964. The relation between the mutagenic and maturation effects remains unclear.

The photodynamic action of proflavine on mature bacteriophage has been studied by HIATT (1960) using T2, and RITCHIE (1964) using T4. These workers have shown that suspensions of bacteriophage absorb proflavine, and the viruses, are rendered photosensitive to visible light as a result of dye exposure.

The work reported here compares the differences in mutagenic effects of proflavine on vegetative T2 wild-type phage to the effects on T2 proflavine resistant mutant phages *pr* and *pr q*. The differences in photosensitivity of the mature progeny from these infected complexes are compared as well. A preliminary note reporting some of these results has already appeared (HESSLER 1964). The work of SCHAFFER (1962) showed that poliovirus propagated in the presence of proflavine are photosensitive, whereas mature poliovirus particles incubated in the dye after normal maturation do not become photosensitive. The experiments in this paper show that T2 wild-type infected complexes are more easily mutagenized by proflavine than *pr* or *pr q* complexes, and that wild-type phage are more photosensitive than acridine-resistant mutant phage produced under the same conditions. A mechanism which causes these differences in dye resistance of T2 wild type and *pr* would be the establishment by the bacteriophage genomes of different capacities for dye uptake into the infected cells. SILVER (1965) has

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shown differential uptake by measurement of fluorescence of acriflavine-treated infected complexes of both T2 wild type and *pr*.

MATERIALS AND METHODS

Bacterial strains: B/1,5 (Luria) cells were used for infected complexes; S (Hershey) cells were used for agar plating as were K-12(λ).

Phage stocks: T2 (Hershey) wild type (acridine sensitive) and *pr*, *pr q*, and *q* (acridine resistant mutants) were used. Occasional use was made of *r*13 (rapid lysis plaque morphology) mutant, as well as *r7*.

Media: Tryptone broth: Bacto-tryptone, 10 g, NaCl, 5 g, distilled H_2O, 1 liter, pH 7.0. Bottom layer agar: Bacto-agar, 11 g, Bacto-tryptone, 10 g, NaCl, 8 g, glucose, 1 g, distilled H_2O, 1 liter. Top layer agar: same as bottom layer, except 7 g Bacto-agar were used. M9 synthetic medium: M9 salts: Na_2HPO_4, 60 g, KH_2PO_4, 30 g, NaCl, 5 g, NH_4Cl, 10 g, distilled H_2O, 1 liter. After autoclaving, salts are diluted 1:8 into sterile distilled H_2O along with 1 part 4% sterile glucose. Final medium has pH of 7.0 and contains .001 M $MgSO_4$. M9 synthetic medium without glucose was used as adsorption medium. Acetate buffer: $NaC_2H_3O_2 \cdot 3H_2O$, 10.9 g, acetic acid, 1.2 ml, distilled H_2O, 1 liter. Dilute 10-fold for use as diluting fluid (pH 5.2). Proflavine (3,6-diaminoacridinium diHCl from Mann Research Laboratories Inc.) was made up biweekly in stock solutions of 200 μg/ml in sterile distilled water. In general, about 1 μg/ml is an inhibitory concentration for wild type, and 4 μg/ml is inhibitory for *pr* and *pr q*.

General methods: Standard methods of assaying and performing growth studies and crosses, plating, and counting were employed (ADAMS 1959). Special techniques are described below.

Mutagenesis: If stocks to be treated were in the r^+ form, the progeny of mutagenized infected complexes were inspected for occurrence of *r* mutants as the index of mutagenic efficiency. When *r7* stocks were used, progeny were screened for r^+ on K-12λ. After adsorption of about five phage per bacterium for 10 minutes at 38°C in M9 medium (without glucose), the infected complexes were diluted into tryptone broth with or without proflavine. After 15 or 16 minutes exposure to dye in the dark at 38°C, the infected bacteria were diluted 100-fold into broth of a noninhibitory dye concentration for an additional 60-minute growth period. Up to 5 μg/ml proflavine was used routinely, and yields of at least 75% of the control were recovered. Growth tube contents were chloroformed to terminate the growth period, and the progeny phage were diluted, plated, and scored for number of *r* mutants among r^+ progeny, or for r^+ revertants when *r7* was the parental stock.

Photoinactivation: In general, growth experiments were performed at dye concentrations in the range of .5 to 5 μg/ml for the first 16 minutes of growth, followed by a 100-fold dilution into plain broth to permit development in a dilute dye concentration. These dilute dye (range: .005 to .05 μg/ml) growth tubes, after chloroform treatment, were further diluted 100-fold into acetate buffer, refrigerated overnight, and photoinactivated the next day. The figure legends explain variations from this general plan. Culture tubes which contained 5-ml samples of the acetate buffer suspensions were exposed to two 4-watt daylight fluorescent bulbs (a substage lamp) at an average distance of 2 cm. This corresponds to a light intensity of about 2000 foot candles, as measured by a General Electric light meter. Samples were assayed at 0, 10, 20, 40, and 80 minutes. The rack of samples and lamp were placed in a refrigerator to prevent excess heating of the samples during the inactivation period. Proflavine samples were kept in red glass tubes or foil-covered tubes when they were not in the complete darkness of the incubator or when not being deliberately photoinactivated in transparent glass tubes.

Phenotypic mixing experiment: An initial cycle involved multiple mixed infection with four phage per bacterium of each parent (*pr* and wild type). During the next cycle, using progeny phage from the first cycle, cells were infected with fewer than .1 phage per bacterium (so that each kind of progeny phage had an opportunity to infect but one host cell apiece) and then grown in proflavine-supplemented broth. The control growth cycle was a low multiplicity of infection (.1 phage per bacterium) of *pr* and wild type separately in proflavine-supplemented broth.

RESULTS

Mutagenesis of wild-type infected complexes: The method of mutagenesis employed was similar to that of DEMARS (1953) and permitted recovery of normal or near normal burst sizes with little loss of infected complexes. Although a more prolonged period of treatment than 16 minutes results in the accumulation of more mutants, there is generally a corresponding decrease in total burst size, so only the short exposures were made.

The most effective period of dye treatment was from 0 to 16 minutes. Proflavine treatment begun later (16 to 26 minutes) in the DNA synthetic period was ineffective mutagenically, probably because of reduced opportunities both for induction of mutations and replication of mutants. Likewise, treatment prior to DNA synthesis(0 to 6 minutes) produced fewer mutants than a 0 to 16 minute treatment (Table 1).

The distribution of mutants among the infected complexes was studied in a single burst experiment to determine what fraction of the mutagenized complexes produced *r* mutants. Many of the productive complexes contained one or more *r* mutants. The average burst size of infected complexes which contained mutants was larger than that of complexes with no *r* mutants. The plates with *r* mutants therefore probably contain most of the double bursts produced in this experiment. It is apparent that the majority of *r* mutants are *rII* type, that is, ones which will not grow on K-12(λ). The remainder of the *r* mutants, which do grow on K-12(λ), may represent *r* mutations at other sites produced by spontaneous mutation during the growth period. Some of the data from this experiment are summarized in Table 2.

Photoinactivation studies: phage grown in dye compared with free phage: The progeny from wild-type infected complexes treated with proflavine were photoinactivated with visible light, as were suspensions of free phage which had been incubated in proflavine, but not formed in its presence. The progeny were inactivated more rapidly than free phage exposed to the same amount of dye (.05

TABLE 1

Period of mutagenic effectiveness of proflavine on T2 infected cells. 2 μg/ml for periods listed, followed by 100-fold dilution into plain broth for 60-minute growth period

Experiment Number	Period of dye treatment	Number of r^+ plaques	Number of *r* mutants
1	no dye	2307	1
	0–16	2876	35
	16–26	1858	2
2	no dye	3336	0
	6–16	4911	36
	16–26	3862	6
3	no dye	1140	0
	0–6	1270	4
	0–16	1169	13

TABLE 2

Single burst experiment: proflavine-treated T2 infected complexes. 2 μg/ml proflavine first 16 minutes of growth; diluted to 0.45 infected cells/tube in plain broth for 60-minute growth period

Summary:

	Number of plaques		Percent	Average
	r^+	r	r plaques	burst size
Untreated (mass lysate, no dye)*	2187	0	<.05	150
Proflavine-treated (single burst)	8069	112	1.38	87

Analysis of single burst:

	No. of plates	Total No. of plaques	Average burst size
0 plaques	168	0	0
1 plaque†	15	15	1
without r plaques	56	4248	76
with r plaques	22	3806	173
Totals	261	8069	...

Distribution of r *plaques:*

rII	r^+	non-*rII*	r^+	mottled	r^+
1	153	1	338‡	1	68§
1	148	1	210	1	94
1	30	1	287	1	180
1	58	1	57	1	73
1	101	1	122	1	187
1	166	1	49	.	...
1	123	2	26	.	...
1	338‡	.	...	.	...
2	236	.	...	.	...
3	68§	.	...	.	...
5	202	.	...	.	...
21	99	.	...	.	...
60	181	.	...	.	...
99	1903	8	1089	5	602

* No single burst of an untreated sample was made.
† Unadsorbed parental phage.
‡ Plate with 1 *rII* type mutant, 1 non-*rII* mutant.
§ Plate with 3 *rII* type mutants, 1 mottled plaque.

μg/ml) for the same time period (Figure 1). Perhaps more dye becomes bound or the dye is more easily bound to photosensitive sites during intracellular growth than when it is deposited through the coat of a mature virus. This treatment-dependent difference in T2 photosensitivity is similar to SCHAFFER's (1962) results with poliovirus—those particles propagated in the presence of proflavine were photosensitive, whereas those incubated in dye after normal maturation were insensitive.

Exposure of pr^+ infected bacteria to .005 μg/ml of proflavine throughout the growth cycle produced progeny phage insensitive to photoinactivation, whereas

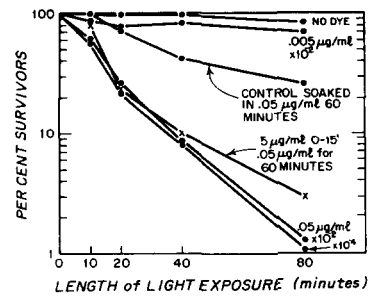

FIGURE 1.—Comparison of photosensitivity of progeny from proflavine-treated infected cells with an untreated free phage suspension which was also incubated in proflavine after completion of maturation. Top curve: normal progeny, not exposed to dye. Below that, the progeny of infected complexes exposed to .005 μg/ml proflavine throughout growth cycle; diluted 10^{-2}, after chloroforming, into acetate buffer. Third curve: part of dyeless progeny were incubated 60 minutes in .05 μg/ml proflavine, diluted 10^{-2} into acetate buffer. Fourth curve (x's): infected cells were exposed to 5 μg/ml proflavine first 15 minutes, diluted 100-fold for 60 min growth at .05 μg/ml, further diluted 10^{-2} into acetate buffer. The two lower curves represent photoinactivation of progeny grown 60 min in .05 μg/ml, then diluted 10^{-2} and 10^{-4}. The data in Figure 1 are from one experiment so that all dye exposures and photoinactivations could be carried out simultaneously.

exposure to .05 μg/ml for a parallel sample of infected bacteria produced photosensitive progeny which inactivated to 1% of the input sample in 80 minutes. Before photoinactivation the progeny gave no evidence of either mutagenic or maturation effects.

Period of proflavine labeling: The extent of photoinactivation appears to be dependent on the quantity of dye present during late developmental stages. The photosensitivity of T2 wild-type progeny grown in the mutagenically innocuous quantity of .05 μg/ml proflavine was the same as that of wild-type progeny grown from 0 to 16 minutes in 5 μg/ml, diluted 100-fold, and then grown the subsequent hour in .05 μg/ml. In each of these cases survivors were about 1% of the initial sample. The dye present during the later part of development becomes irreversibly associated with the final mature phage particles, since the same photoinactivation curve was obtained whether the dye concentration of the final lysate, after being chloroformed, was diluted 10^{-2} or 10^{-4} (two bottom curves, Figure 1).

Experiments were performed specifically to test whether proflavine added early and reduced by dilution before DNA synthesis began could also be detected in the progeny phage. Whether proflavine was added prior to DNA synthesis (0 to 6 minutes) or was present when DNA synthesis was going on (0 to 16 minutes), progeny were recovered which had essentially similar survival rates consistent with the concentration of dye present after a 100-fold dilution into broth for completion of the growth period. Other experiments have shown that proflavine added late (16 to 26 minutes) was also incorporated into phage, and these were inactivated to the same extent as those phage which underwent early dye treatment. Thus infected bacteria are permeable to dye throughout the growth cycle but progeny phage acquire dye label corresponding to the quantity present

during the completion of maturation. Later on, data will be presented which suggest that the cells infected with *pr* or *pr q* acquire dye from the medium at a slower rate than cells infected with wild type.

The extent to which progeny phage are photoinactivated does not imply that complexing of dye with DNA during DNA synthesis is essential for the binding of dye which is photosensitive. Indeed the dye which is responsible for the photoinactivation effect may be associated with protein structures of the mature phage.

Effective mutagenic concentration of proflavine: In a series of mutagenesis experiments, T2 wild-type progeny were photoinactivated after treatment of infected complexes with a series of different proflavine concentrations for the initial 16 minutes of growth. (The usual 60-minute growth period at a 100-fold dilution was then permitted.) For example, in one experiment, mutagenesis was essentially the same whether .5, 1, or 8 μg/ml proflavine were initially added (percent *r* mutants: .86, 1.11, and .86, respectively; dyeless control sample was .03% *r*). But the photoinactivation rates of the dilute growth tube contents reflected the different quantities of dye added in that the progeny from the 1 μg treatment were inactivated twice as rapidly as those from the .5 μg sample and about ten times more slowly than progeny of the 8 μg treatment. That the dye was bound irreversibly was demonstrated when 10^{-4} dilutions of each growth tube after burst gave the same photoinactivation results as the usual 10^{-2} dilution. There appears then to be a limit to the amount of proflavine which is effective mutagenically under the conditions of these experiments. There is in effect a plateau reached where raising the concentration of proflavine does not increase the induced mutagenesis.

Comparison of mutagenic sensitivity of T2 wild-type and T2 acridine-resistant mutants: One μg/ml of proflavine (and on occasion, .5 μg/ml, as above) for the first 16 minutes of growth was sufficient to induce at least a 20-fold increase in *r* mutants in the final progeny population of either wild-type or *q*-infected complexes. When *pr q* and *pr* complexes were mutagenized, approximately four times more proflavine was needed to induce 20-fold more *r* mutants than was needed for cells infected with wild type or *q* (Table 3). These experiments support the idea that the intracellular dye content achieved in 1 μg/ml proflavine was higher in T2 wild-type infected complexes than in *pr* or *pr q* complexes. Only by raising the dye concentration to 4 μg/ml for *pr* and *pr q* were mutagenesis results obtained which were similar to wild type and *q* at 1 μg/ml. STREISINGER *et al.* (1961) have found no difference in mutagenic capacities of T4 infected complexes using *ac* and ac^+ phages; but no data were presented which permit a comparison with the range of concentrations tested here in T2.

The increase in r^+ revertants above background levels differs according to whether $r7pr^+$ or *r7pr* was the infecting phage. As was the case above, the pr^+ form of *r7* was more sensitive to lower proflavine concentrations than the *pr* form of *r7* (Table 4).

Comparison of photosensitivity of pr *and* pr q *progeny with wild type:* A series of photoinactivation experiments were performed to test for quantitative differences in photosensitivity of progeny of infected complexes which had undergone

TABLE 3

Comparative proflavine mutagenesis resulting from parallel treatments of acridine sensitive and resistant complexes

Genotype	Proflavine concentration (μg/ml)	Number of r^+ plaques	Number of r plaques	Percent r mutants ± SE	Number of experiments
Wild type	0	36406	13	.034 ± .016	7
	1	1470	15	1.02	1
	2	14053	139	.99 ± .25	3
	4	23047	294	1.27 ± .42	3
	5	2357	41	1.74	1
q	0	6542	1	.015 ± .004	2
	1	1906	18	.95	1
	4	2659	23	.86	1
	5	1499	24	1.60	1
pr	0	12300	3	.024 ± .004	4
	1	3170	8	.25	1
	2	11255	31	.26 ± .08	2
	4	2789	11	.43	1
	5	5930	77	1.30	1
pr q	0	25327	2	.008 ± .001	4
	1	4690	0	<.02	1
	2	7831	9	.12	1
	4	6414	39	.61 ± .30	2
	5	15049	45	.30	1

Increase in r:r^+ ratio is index of mutagenic efficiency. All proflavine treatments were from 0 to 16 minutes followed by 100-fold dilution to reduce dye concentration during additional 60-minute growth period.

TABLE 4

Production of r+ *revertants by* r7pr+ *or* r7pr-*infected cells**

Genotype	μg/ml proflavine	Frequency of r^+†	Number of experiments
r7pr+	0	1.0 ± 0.6	4
	1	9.8 ± 1.3	4
	2	18.0 ± 1.5	4
	4	38.0 ± 6.0	4
	8	43.0 ± 10.0	2
r7pr	0	5.3 ± 1.4	6
	1	7.4 ± 2.6	6
	2	14.0 ± 3.3	6
	4	31.0 ± 6.7	8
	8	53.0 ± 1.5	2

* Treatment procedure same as in Table 3. In each experiment 400 to 1000 r plaques were counted on S; and 50 to 100 r^+ plaques were counted on K-12(λ).
† Frequency × 10^{-7} ± standard error of the mean.

similar dye treatment. The experiments showed that *pr*-infected complexes gave evidence of lower proflavine uptake than cells infected with wild type (or *q*), unless the proflavine concentration was above 4 μg/ml. There appears therefore to be a difference in "threshhold" of infected complexes to dye uptake, and this difference is mediated by the phage genotype.

In general, progeny from proflavine-treated infected complexes of either *pr* or *pr q* were photoinactivated at the same rate, but a significantly slower rate than progeny from wild-type infected complexes or *q*-infected complexes (except at high dye concentrations, which will be discussed below). A possible explanation of these differences is that dye penetrates *pr*-infected cells more slowly than wild-type infected cells. When a small amount of dye, .05 μg/ml, was present throughout the growth cycle, the subsequent *pr* progeny were photoinactivated more slowly than were wild-type progeny, which were inactivated to about 1% (as mentioned earlier). Another example of these postulated different uptake rates is suggested by this experiment: When the dye concentration was initially high, 2 μg/ml, but diluted 100-fold to .02 μg/ml for the remainder of the growth period, both *pr* and *pr q* progeny were less photosensitive than wild-type progeny so treated (Figure 2). This differential sensitivity disappeared, however, as dye concentrations were raised, and a 0 to 16 minute treatment with 5 μg/ml proflavine (followed by 100-fold dilution into broth for 60 minutes) yielded *pr* and wild-type progeny which were equally photosensitive (Figure 3). The point at which rates of photoinactivation of acridine-resistant progeny and wild-type progeny were not significantly different from one another corresponded to a 16-minute treatment with 4 to 5 μg/ml for *pr* and *pr q* compared with 1 to 2 μg/ml for wild type (Figure 3). These levels correlate roughly with mutagenically effective doses of proflavine—amounts which induce approximately equal increases in *r* mutants in *pr* (or *pr q*) and wild-type (or *q*) infected complexes, respectively (Table 3).

Photoinactivation of progeny from mixedly infected cells: The progeny of proflavine-treated mixed infections of *pr* and wild type or *pr q* and wild type gave an intermediate rate of photoinactivation—slower than wild type, faster than the acridine mutants. All classes of progeny of the mixedly infected cells were inactivated to the same extent. Hence there was no intracellular selection among proflavine-treated complexes, which favors production of phage with *pr* in the genome when cells were infected with both *pr* and wild-type (pr^+) phages.

Phenotypic mixing experiment: If the mechanism by which proflavine uptake is controlled is an early one, it may be determined initially by some function of the external structure of the phage—some difference induced in the cell wall, for example, upon attachment of the phage. Therefore, an experiment was designed to look for phenotypic mixing.

One expects the progeny of a mixed infection to be phenotypically mixed with respect to external phage-structural-components (STREISINGER 1956). If a factor which controlled proflavine uptake by infected cells resided in the external coat of the phage particle, then the extent to which infected cells acquire dye may depend on the external structure of the infecting phage: *pr* genome + wild-type

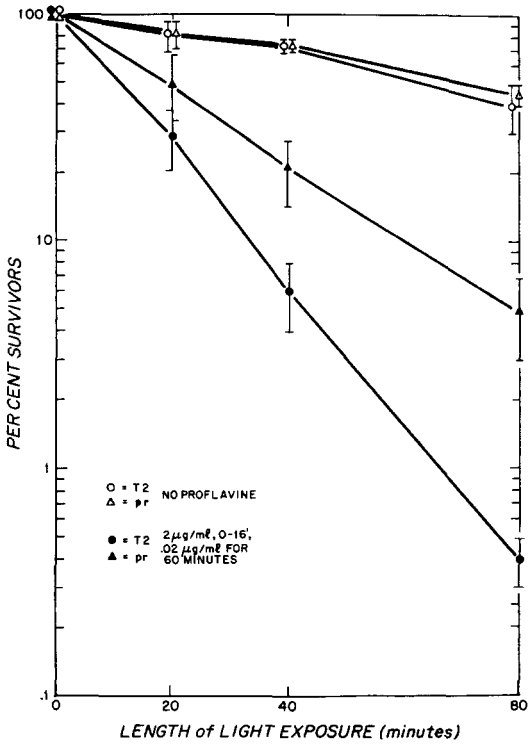

FIGURE 2.—Comparative photosensitivity of progeny of T2 wild-type and *pr* infected complexes. ○ = T2 wild type, no dye; △ = *pr*, no dye. ● = T2 wild type, and ▲ = *pr*, each treated with 2 μg/ml for the first 16 minutes of growth period, diluted 100-fold to .02 μg/ml for 60 minutes more. $CHCl_3$ added, and all diluted 10^{-2} into acetate buffer. The points for wild type (labeled T2 in the figure) represent 6 experiments. Nondye curve for *pr*, 4 experiments; proflavine curve, 5 experiments. Extent of standard error of mean indicated by brackets.

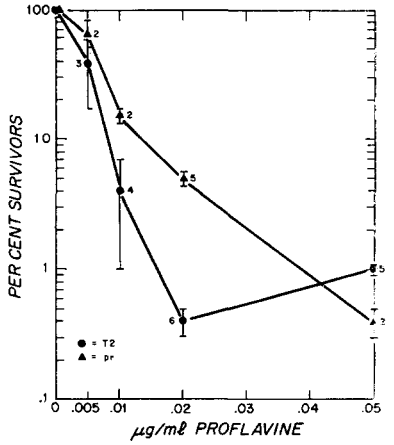

FIGURE 3.—Percent survivors after 80 minutes of photoinactivation. Numbers next to symbols = number of experiments. Range of proflavine concentrations tested was .5 to 5 μg/ml for first 16 minutes, then 100-fold dilution to the quantities indicated in graph. After 60 minutes growth, chloroform was added, and contents of growth tubes diluted 10^{-2} into acetate buffer. The inactivation curves leading to the 80-minute survival data presented here all followed first order kinetics of the sort presented in Figure 2. Standard error of mean indicated by vertical lines. ● = T2 wild type. ▲ = *pr*.

coat = high dye uptake by infected cells and abnormally photosensitive *pr* progeny; pr^+ genome + *pr* coat = low dye uptake by infected cells and abnormally photoresistant pr^+ progeny.

A standard cross was performed between *r*13*pr* and wild type (r^+pr^+). Afterward only the recombinant classes *r*13pr^+ and r^+pr were considered in the analysis because these came from cells which received both parental types and therefore might show phenotypic mixing. Progeny from the cross were adsorbed singly onto cells for another cycle of growth in dye (.05 μg/ml proflavine). Phage growth was interrupted with the addition of chloroform at 15 minutes, albeit the burst sizes were small (about 20) to avoid the complications of reattachment of early-lysing progeny to residual host cells. The progeny of this dye treatment

were then photoinactivated; ten times as many r^+pr survivors as $r13pr^+$ survivors were found. Ten percent of the *pr* progeny, and 1% of the pr^+ progeny survived photoinactivation.

Control growth-tubes of *pr* and pr^+ grown separately at low multiplicities of infection in .05 μg/ml and then mixed and photoinactivated together gave the same relation between survivors of photoinactivation as was found in the case of the r^+pr and $r13pr^+$ recombinants just mentioned. The absence of phenotypic mixing means that the *pr* gene does not code for a coat protein.

DISCUSSION

These experiments suggest that *pr* or *pr q* infected complexes take up less proflavine than do wild type (or *q*) infected complexes, except at high dye concentrations. This difference offers an explanation for many results obtained in earlier experiments (HESSLER 1963). Both the inefficiency of proflavine mutagenesis and the photoresistance of progeny of treated complexes of *pr* probably results from a lower intracellular dye concentration than was present for wild-type phage grown under the same conditions. Support for the idea of differential dye uptake is lent by the work of SILVER (1965) which was performed concurrently in another laboratory. He found by measuring dye concentration after centrifuging infected and uninfected cells that T2 wild-type infected cells took up significantly more acriflavine than T2 *pr* or *pr q* mutant infected cells, and that uninfected cells took up the least quantity of acriflavine. We have confirmed this result in our laboratory (unpublished data). The experimental problem raised by the hypothesis of differential uptake is to find a mechanism by which T2 infecting phage interact with the host cell to alter the permeability of the infected cell. It would seem that infected cells become permeable for transport of metabolites in or out of the cell and that the transfer of acridines reflects some accident of their size, net charge, or structure.

A major problem which remains is what the proflavine does once it is inside the infected cell, and there is still no solution for this. Perhaps only a limited amount of dye taken up by the cells becomes involved in mutagenesis. When an excess of proflavine is added over what induces a significant increase in *r* mutants (about 1 to 2 μg/ml for wild type; 4 to 5 μg/ml for *pr* or *pr q*), this excess is reflected in a more rapid rate of photoinactivation but by little increase in mutant level. These *in vivo* data may reflect some aspect of the *in vitro* complexing of proflavine with DNA molecules, which seems to be expressed in at least two ways (BEERS, HENDLEY, and STEINER 1958). The mode of proflavine binding which induces mutation by perhaps causing addition or deletion of bases is thought to be intercalation between base pairs of the DNA molecules (LERMAN 1961, 1963, 1964; ISENBERG *et al.* 1964). At the time of proflavine treatment (0 to 16 minutes), there are a limited number of phage DNA molecules available per infected complex. This would tend to limit the amount of the dye which could be effective mutagenically. The residual proflavine whose presence we later infer from the photoinactivation studies may represent the dilute remainder of the

input proflavine. This residual proflavine may be bound to DNA of the progeny phage in a manner different from the mutagenic proflavine which acted on the parental phage DNA and replicating DNA. Also the proflavine measured by photoinactivation may be bound to protein structures of the mature phage. A unified explanation for how the presence of proflavine brings about interference with mutation and maturation remains elusive.

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SUMMARY

Progeny phage from infected cells formed from acridine sensitive wild-type T2 or the acridine resistant mutants *pr* and *pr q* in the presence of proflavine showed significantly different proflavine-induced mutation rates and photosensitivity. This has led to the conclusion that T2 wild type infected complexes take up more proflavine than do those of *pr* or *pr q*. By raising the dye concentration about fourfold, however, the effects on T2 wild type infected cells can be duplicated in the acridine resistant infected cells.

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