TECHNICAL PAPERS

Ultraviolet Action Spectrum of T1 Bacteriophage

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T1 bacteriophage is a self-duplicating organism of remarkable stability. In the presence of a small amount of dried broth it can be maintained dry almost indefinitely. Advantage has already been taken of this to study the target size for deuteron inactivation (*5*). This target appears to be smaller than the whole bacteriophage particle. It is therefore of some interest to study the relative effectiveness of ultraviolet light in causing inactivation. Such studies have been made by Gates (*1*) on *S. aureus* bacteriophage and by Hollaender (in *3*) on various bacteria. A recent review of the significance of ultraviolet action spectra has been given by Loofbourow (*3*).

If the number of bacteriophage particles initially present is n_0 and after receiving an intensity I for a time t a number n survive then the relation

$$\frac{n}{n_0} = e^{-aIt}$$

holds where a is a constant for any one wavelength. This relation requires the random absorption of photons and applies only if the bacteriophage layer is uniform, the ultraviolet light is uniform, and there is no absorption by broth. Results indicate that if allowance is made for departure from these conditions, the relation is obeyed.

Our procedure has been to measure a for a series of wavelengths of light given by a quartz mercury arc and the resulting plot of a versus wavelength is the action spectrum.

Bacteriophage assays are made by plaque counts, the bacteriophage being mixed with *E. coli* and agar sufficiently warm to spread out on a Petri dish. The agar, coli, and bacteriophage mixture is then incubated at 25° C for about 8 hr and the plaques counted. We are indebted to Mrs. Marjorie Reaume for almost the whole of the preparation and plating work.

Selection of a particular wavelength was by means of a monochromator built according to a design by Harrison (*2*). It consists of an aluminized concave mirror placed at a slant under a water surface. A U-A2 uviarc is the principal light source used and the intensity of the emergent beam is monitored with a photocell which has been calibrated against a thermopile.

The results were compiled from seven runs, during which time some modifications of equipment and technique were made. The spectral dependence was found to be similar in spite of change of arcs, renewal of the mirror surface, and change of the amount of dried broth in which the bacteriophage was irradiated. Full energy calibration was only carried out in the last few runs.

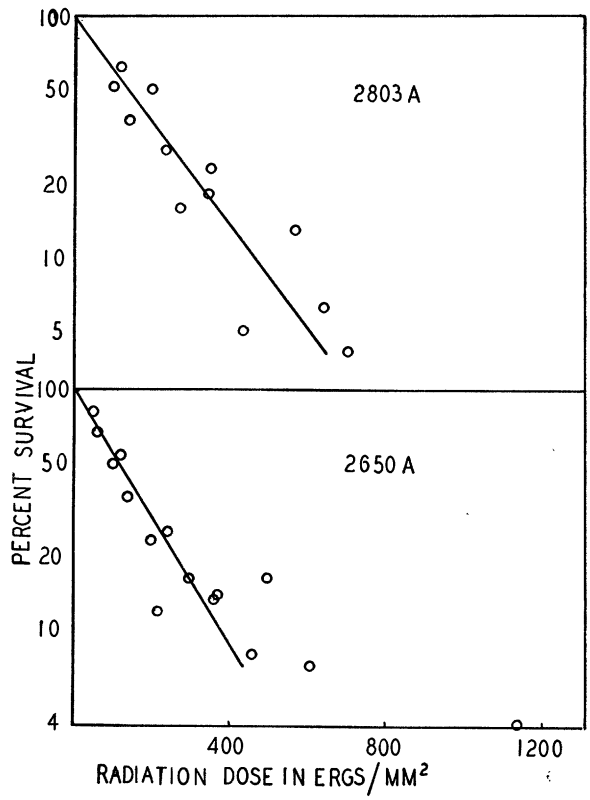

FIG. 1. Representative inactivation curves in which the logarithm of the percent survival is plotted against the radiation dose. The logarithmic relation holds over a factor of ten. At shorter wavelengths absorption in the broth, combined with sample inhomogeneity, causes a departure at high dose figures.

In order for a to be a valid indication of sensitivity, the rate of inactivation with energy dose must follow the relation given. Unless irradiation is at the same intensity for all wavelengths, the validity of doses as products of intensity and time must also be considered. Curves are shown in Fig. 1 for wavelengths 2650 A and 2803 A. Data are included for three runs, for which the intensity was varied over a factor of 2.5. Within the dispersion of the data, reciprocity of time and intensity appears to be acceptable. The dispersion for these runs is at least partly statistical, since control plaque counts were about 100. Treatment of the data as logarithmic also appears valid, at least to the 37% point at which a is determined. At longer wavelengths individual curves were even more accurately logarithmic.

At 2650 A and shorter wavelengths a positive curvature at lower survivals is noticeable. Decrease of the amount

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of broth on each irradiation strip from .05 to .01 ml reduced this curvature from 20 or 30% survival to below 10%. Absorption in the broth film is the most probable explanation, for although an absorbing film of uniform thickness would still give nearly logarithmic inactivation (*4*), we were not able to obtain consistently uniform broth layers. The difficulty of measurement and transfer of these small volumes is the most likely cause of the dispersion of the points on our inactivation curves.

TABLE 1

λ, A	3664	3131	2967	2894	2803	2760	
37% dose, ergs/mm²	~100,000	30,000	1200	750	220	224	
λ, A	2700	2650	2540	2482	2400	2350	2250
dose	207	185	232	455	523	327	690

The calibration of intensity in absolute units must be considered less accurate than the relative intensities of various wavelengths. The energy doses in ergs/mm² for reduction to 37% survival are shown in Table 1. The action spectrum plot is given with *a* calculated for *It* expressed in quanta/mm².

The results are very similar to those obtained by Gates. The features to note are the minimum at 2425 A, which is rather long in wavelength for desoxyribose nucleic acid, and the rather high inactivation at 2800 A. The value of *a* appears to fall at 2250 A, but this may be apparent and not real, since there can be heavy broth absorption. We do not believe that there is a sharp rise corresponding to protein absorption, however, as the broth absorption as estimated by rough measurements could hardly have exceeded a factor of two.

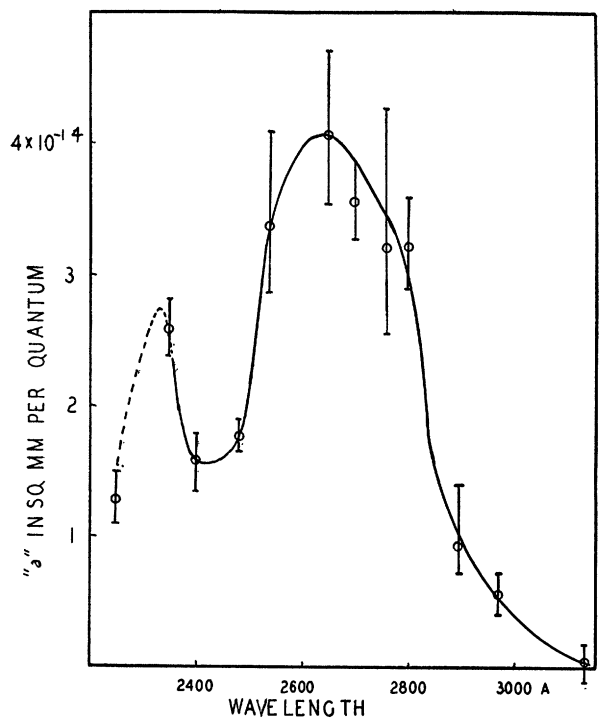

FIG. 2. Relative effectiveness of various wavelengths of ultraviolet light in causing inactivation of T1 bacteriophage.

A comparison with work on bacteria as tabulated by Hollaender can be made. Our figure for the 10% dose at 2540 A is 535 ergs/mm². This shows that dried T1 bacteriophage is less susceptible at this wavelength than vegetative bacteria, but more so than spores.

References

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Lysis of Formalinized Bacteria by Bacteriophage

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Recently it has been pointed out (*1, 2*) that many lysed cells are found in mixtures of young and actively growing cultures of the B strain of *E. coli* with suspensions of certain bacteriophages when such mixtures are chilled and prepared for electron microscopy after very short incubation. After longer incubation stages in the development of new bacteriophage, particles are evident in such masses of lysed protoplasm. We have now found that the same phenomena of bacterial lysis and bacteriophage production are observed using bacteria previously inactivated with formaldehyde.

The bacteria used were 1 to 1½-hr cultures in tryptose broth. They were inactivated by being held for 15–30 min at room temperature after the addition of the desired volume of commercial formalin. Then the cells were washed once or twice by putting them in fresh broth and sedimenting them in a centrifuge. The washed suspension was made up to have a turbidity approximately that of the original culture. Effectiveness of the formalinization was tested in each instance by streaking a loop of this suspension on an agar plate and incubating. Such test plates remained completely free of bacterial growth when a concentration of 0.2% or higher of formaldehyde was employed; a few isolated colonies sometimes developed if the formaldehyde concentration was 0.04 or 0.08%. Such suspensions of killed, washed bacteria become clear on incubation following the addition of a suitable bacteriophage suspension containing enough particles to infect the bacteria present. Samples for electron microscopy were withdrawn after periods of incubation up to 1 hr, immediately chilled in ice water, and centrifuged cold, and the sediment was washed by successive centrifugations first from 0.5% formalin-saline and then from water-formalin. Final suspensions in a small volume were spread and dried in the usual formvar-covered grids and