Absence of Photoreactivation in T1 Bacteriophage Irradiated with Ultraviolet in the Dry State[1]

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Photoreactivability of bacteriophages, following ultraviolet irradiation, has been shown (*1*) to depend on adsorption to the host bacterium and on factors influencing the state of the phage-bacterium complex, such as the metabolic state of the host, temperature, etc. The present paper deals with the finding that the state of the phage itself, at the time of exposure to UV, has a very marked effect on the subsequent reversibility of the damage.

Bacteriophage T1, as already reported (*2*), is stable in the dry state. In our experiments, uniform dry preparations were made by spraying from an all-glass commercial penicillin nebulizer. The phage was collected on glass cover slips, 18 mm in diameter. The cover glasses were supported in circular depressions, provided at the periphery of a rotating platform, so that 8 could be prepared at once. The spraying was done inside a cardboard box.

Suspensions of T1 in broth and in 2% ammonium acetate (*3*) were used for spraying. The latter were prepared by centrifugation of a broth stock and resuspension of the phage in the new medium. Assays of the dried phage were carried out by dropping the cover glasses into 5 ml of broth, diluting further in broth if necessary, and plating by the Hershey agar-layer technique (*4*). In experiments requiring a determination of the recovery of phage after drying, Na^{24} was added to the phage suspension, which was to be sprayed, in a final concentration of about 5 μc/ml. (The injury to the phage due to β- and γ-radiation is far too small to affect the results.) The dry samples were counted with a Geiger counter, along with a standard reference preparation obtained by pipetting 0.02 ml of a 1/100 dilution of the phage-Na^{24} suspension onto a cover glass and allowing this to dry at room temperature. From the ratio of radioactive counting rates and the phage titer of the original suspension, it is a simple matter to calculate the volume sprayed onto each coverslip (10^{-3}–10^{-4} ml) and the total number of phages in a given preparation. Although these preparations will be referred to as "dry" in what follows, it is of course possible, and indeed likely, that the phage retains some water of hydration that is not removed by this procedure.

Phage T1 is as stable in 2% ammonium acetate as it is in broth. Spraying of a broth suspension yields better than 90% recovery of viable, dry phage and is quite reproducible. However, spraying of an acetate suspension results in only about 10% recovery and, although different cover glasses show the same counting rate, and hence the same total number of phages, the viable titers may vary by as much as a factor of 2 or 3. Therefore, when it was desired to run a radiation survival curve on these preparations, several determinations had to be averaged to obtain significant data for each point. In spite of these limitations, the acetate-sprayed dry preparations have an advantage in that the suspension medium is completely evaporated, as shown visibly and in electron micrographs.

The source of UV was a 15-w G-E sterilamp, and the sample distance from the center of the lamp was 56 cm. Photoreactivation was carried out by the plating method (*1*)—i.e., by immediate exposure of the phage plated with a large excess of *Escherichia coli*, strain B, to the reactivating light. This was provided by an H-5 lamp, filtered to give a band peak at 3650 A, the most efficient wavelength for this system (*1*). The lamp was 1 ft from the agar plate, and a 1-in. layer of .05 *M* Cu Cl_2 was placed between the lamp and the plate, to minimize heat inactivation. The time for maximum photoreactivation was determined to be ½ hr, but all plates were routinely exposed for 1½ hr before incubation in the dark. A comparison set of platings was carried out in the dark, followed by immediate incubation.

Comparison studies of T1 irradiated by UV while in solution were done according to established methods (*5*). A broth stock was diluted 100-fold in either Mg-saline buffer[4] or ammonium acetate, and 5 ml irradiated in a 10-cm Petri dish, at the same distance from the sterilamp, with constant agitation.

The result of irradiating T1 in the dry state is that, within the limits of experimental error, the damage is no longer photoreactivable. This is shown in Fig. 1 for phage prepared by spraying a broth suspension (open circles and squares). Similar data were obtained for phage prepared from an acetate suspension. Comparison curves for the irradiation of T1 in Mg-saline buffer are also shown (full circles and squares). It is to be noted that the sensitivity to UV is not affected.

Table 1 shows that this loss of photoreactivability occurs only if the phage is dry at the time of UV irradiation. Irradiation of the phage in solution followed by drying does not result in loss of photoreactivability. The same is true for drying of the phage, followed by resuspension and irradiation.

The figures in Table 1 might suggest that dried phage is more sensitive to UV, since 60 sec of UV inactivate dry phage by a factor of about 50, whereas the titer in acetate drops by a factor of about 20. This discrepancy, however, might be ascribed to some sort of protective mechanism acting in the acetate suspension. If phage is irradiated in buffer, the factor is 50

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[4] Na Cl, 0.15 *M*; Mg SO_4, 10^{-3} *M*; Na_2HPO_4, 10^{-2} *M* (pH adjusted to 6.5).

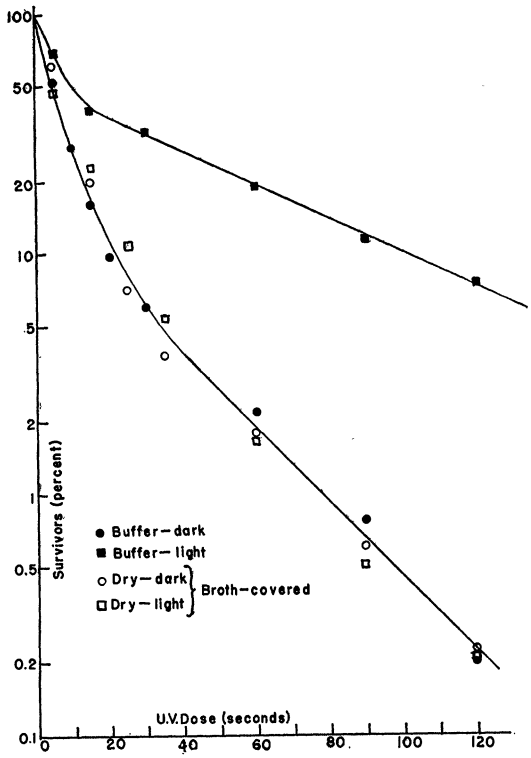

FIG. 1. UV inactivation (dark) and photoreactivation (light) of T1 irradiated in buffer solution and in dry preparations made by spraying a broth suspension.

also. There seems to be no reason to assume that the survival in the dry state should be compared with the acetate suspension rather than the buffer suspension, since drying leads not only to evaporation of water, but also to sublimation of ammonium acetate. It has not as yet been determined whether the lowered sensitivity in acetate is due to UV absorption in this medium or to some protective effect.

The experiments were carried out at room temperature. The effect also persists, however, at lower temperatures. This was shown by experiments in which the cover glass preparation was supported inside a watertight cell supplied with a quartz ceiling. The cell was filled with Drierite and kept in a water bath at 5° C for ½ hr before irradiation, in order to ensure temperature equilibrium. No photoreactivation was detected either for the phage irradiated at 5° or at 25°. The effects of temperatures above 25° C are under study.

Since the UV survival curve is unchanged in the dry state, it seems likely that inactivation proceeds by the same mechanism. It would appear, however, that photoreactivation of some of this damage requires not only adsorption to bacteria and application of light but is also dependent on the state of the phage at the time of UV exposure.

TABLE 1*

EFFECT OF DRYING ON PHOTOREACTIVATION OF PHAGE

Treatment	Assay	Ratio (UV): (UV → PR)
A	1.0×10^{10}	
A → UV	5.0×10^{8}	1: 6.2
A → UV → PR	3.1×10^{9}	
A → D	1.3×10^{9}	
A → D → UV	2.8×10^{7}	1: 1.4
A → D → UV → PR	3.9×10^{7}	
A → UV → D	8.8×10^{7}	1: 5.6
A → UV → D → PR	4.9×10^{8}	
A → D → A → UV	8.3×10^{7}	1: 6.3
A → D → A → UV → PR	5.2×10^{8}	

* *A* refers to suspension (also resuspension) of T1 in 2% ammonium acetate, *D* to drying, *UV* to a 60-sec exposure to the germicidal lamp, and *PR* to exposure to the H-5 lamp of sufficient duration to obtain maximal reactivation. All data are normalized to an initial titer of 1.0×10^{10}.

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Greater Resistance of the Female to Experimental Burns Following Starvation

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In the course of experimentation in the chemoprophylaxis of burns (*1, 2*), certain trends in the survival rates of intact rats subjected to thermal injury were observed that could not, under the conditions described, be unequivocally attributed to sex factors. In order to investigate sex differences under more rigorous conditions, starvation was selected as an additional stressor prior to burning. Since it has long been known that liver lipids increase during fasting (*3*), and, more recently, that under fasting conditions the lipid accumulation is higher in the liver of the female than in that of the male (*4*), it was hoped that the marked lipotropism seen in the starved female would be a factor in increasing the female survival rate over that of the male after burns. Of the lipotropic substances, only methionine has to our knowledge been tested in burns and found to be protective (*5*). Accordingly, each of three groups of albino rats consisting of nearly equal numbers of males and females of similar body weights was starved for 50, 75, or 100 hr, anesthetized with pentobarbital sodium, immersed up to the neck in water at 75° C for 20 sec, according to the method of Hazán and Treadwell (*5*), and returned to a standard diet. Water was available