Survival of T4 Bacteriophage Irradiated in Suspension by Protons at Energies between 4.6 and 2.5 MeV. Dependence on Linear Energy Transfer (*).

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Summary. — The mean survival doses of T4 bacteriophage irradiated in suspension by protons were measured at six values of incident energy. Due to the thickness of the target, the beam stopped inside the suspension, so yielding the overall mean dose, averaged from the incident energy to zero. By a suitable analysis of the overall mean doses, measured at different incident energies, the mean doses in narrow intervals of energy, which may be related to definite LET values, were extracted.

PACS. 87.50.Gi. – Ionizing radiations (UV, X-ray, γ-ray and particle radiation effects).

1. – Introduction.

In a previous paper (1) we measured the lethal damage induced by protons of 3.7 MeV in a suspension of T4 phages. Since the proton beam was stopped in the suspension, we were able to determine the value of T4 radiosensitivity averaged over the energy between the incident value and zero.

It is known that an important parameter involved in radiation damage is the linear energy transfer (LET). Since it is not possible to irradiate the T4 in a dry form so as to have a thin target, we applied a series of irradiations at different

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incident energies. In this way it was possible to separate the contributions deriving from protons of different LET by a suitable analysis of the results.

The present paper contains the description of the experimental method and the measured values of the survival sensitivity of T4 phage irradiated by protons at six incident energies between 2.5 and 4.7 MeV. The method used appears to be appropriate for the measurement of damage to viruses in suspension.

2. - Experimental procedure.

2'1. Apparatus. - Irradiation was effected with protons from an electrostatic tandem accelerator (3 MV at the terminal). The phage suspension, contained in a small well, flowed continuously in a circuit under the action of a peristaltic pump. At every circulation round the suspension passed through the beam as a layer (0.3 mm thick) confined between two kapton foils. The time to accomplish one complete round was about 30 s. The beam intensity during irradiation was about 5 nA. Details of the experimental layout are reported in ref. (1).

2'2. Phage propagation. - E. coli B cells, grown at 37°C with aeration in M9 medium to 2 \cdot 10^8 cells/ml, were infected in the presence of L-tryptophan by T4D wild-type phage at a multiplicity of infection of 0.01. Lysis of the cultures was normally attained within 4 h and T4 lysates were subsequently purified following standard procedures (2).

2'3. Irradiation and viability measurement. - Before irradiation, the phage stocks were diluted into nutrient broth (NB) (Hershey preparation: 0.8% Bacto-Nutrient Broth, 0.5% Bacto-Peptone, 0.1% glucose and 0.5% NaCl) and the apparatus was filled with 3 ml of phage suspension (10^{11} phages/ml). At each dose a sample of 50 \mu l of phage suspension was withdrawn and was immediately diluted into 500 \mu l of NB to avoid possible aftereffects (3). Before drawing, the liquid was totally sucked into the well to ensure complete mixing. Plaque-forming ability was measured by standard plating. Bottom and top agars contained 10 g/l of Bacto-Tryptone plus 20 and 7 g/l of Bacto-Agar, respectively.

3. - Measurement of the mean dose.

The circulation of the phage suspension permits uniform irradiation even if the protons are stopped inside the fluid. Under the hypothesis that after every circulation round the irradiated and nonirradiated parts of the suspension are


(2) D. FREIFELDER: *Virology*, 36, 613 (1968).