STUDY OF THE RADIOSENSITIVE STRUCTURE OF T2 BACTERIOPHAGE USING LOW ENERGY ELECTRON BEAMS

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ABSTRACT Hydrated T2 bacteriophage were irradiated with 0.75 to 90 kev electron beams. A thin foil isolated the sample chamber from the electron gun source. Survival (plaque formation) was observed. Apparent cross-sections and D_{37} doses were determined. The maximum cross-section of about 5×10^{-8} μ^2 is roughly equal to the cross-sectional area presented by the phage core. As beam energy was increased the average D_{37} dose first attained a minimum value of about 23 kr for 1 kev electrons (which penetrate the relatively inert protein coat) after which the average D_{37} dose rose with beam energy to a maximum value of about 50 kr for fully penetrating beams. These dependencies suggest that the radiosensitive structure exists as a peripheral shell rather than a uniformly sensitive core. A tentative model for the phage structure, based on this and other evidence, is presented.

INTRODUCTION

Low energy electron beams have been utilized in vacuum by Davis and Hutchinson (1), Davis (2), McCrea, Preiss, and O'Loughlin (3), and Preiss and Pollard (4) to deliver energy to various depths in biological material. Such studies have been utilized to estimate thicknesses of spore and viral coats and the location and configuration of nuclear or enzymatic material.

A technique, utilized in work reported here, enables similar studies to be carried out with hydrated specimens (5-7).

TECHNIQUE AND APPARATUS

Fig. 1 illustrates the essential features of the irradiation technique. Electrons originating at a tungsten silicate filament are accelerated across a cathode-anode gap of from 2 to 12 mm and pass through a beam collimator slit (0.25 mm × 2 mm) into either a beam measuring system (*in vacuo*) or the biological sample. A collodion isolation foil of about 0.75 $\mu g/cm^2$, mounted on the collimator slit, separates the gun vacuum from the lower chamber. The collimator current is used as a reference for setting the beam current during irradiations.

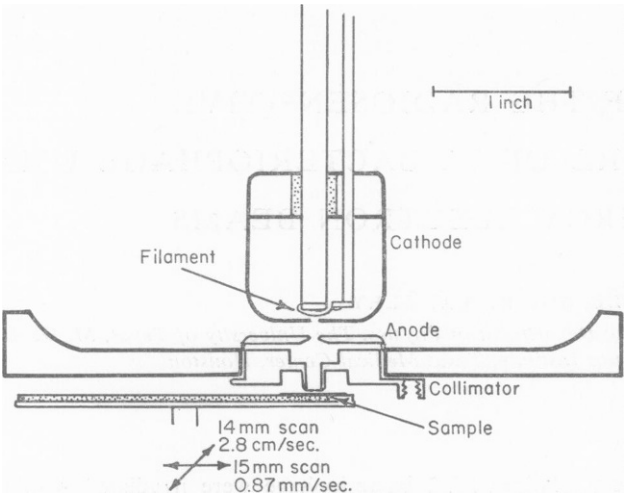

FIGURE 1 Experimental arrangement for low energy electron beam irradiations.

5 to 10 μl of a phage suspension in 1 per cent broth were pippetted onto the surface of 0.5 inch diameter Millipore (10 mμ porosity) or Gelman polypore (4 mμ porosity) membrane filters which were placed on broth-soaked underpads. Surplus fluid passed through the filters leaving the phage particles exposed on the surface. The sample and mounting systems were precooled and maintained at about 2°C during the irradiation. A mechanical (raster) scan system provided for beam coverage of a 0.5 inch square area during a 17 second interval. Each point on the specimen passed perpendicularly under the beam slit about five times during a complete raster. This large degree of beam overlapping, in combination with scattering by the foil and water vapor, was considered sufficient to provide a uniform exposure over the sample. Although the beam distribution was not directly measured, an exponential survival-dose response for phage particles, *Escherichia coli* cells, and *Streptomyces* T12 spores through two or more decades (for fully penetrating electrons) implied a uniform distribution. Gamma irradiation of these materials also produced exponential responses. Non-uniform exposures tend to distort exponential responses to produce "tailing" or a concave upward bending of the log survival *versus* dose curves. The "tailing" which occurred at lower voltages was considered caused by partial shielding and is discussed later.

The filter surface could be placed within 1 mm of the isolation foil. Water vapor remaining after air evacuation of the lower chamber produced a partial pressure of 6 mm Hg as measured with a mercury manometer. This vapor absorption thickness corresponds to about 0.7 μg/cm^2 of collodion, thus the preabsorption path caused by the isolation foil and water vapor was about 1.5 μg/cm^2. Samples could be inserted, pumped down, irradiated, and removed in a 1-minute period. Following irradiation the filters were placed in 2 to 10 ml of 1 per cent broth and the phage released from the filter by sonication for 1½ minutes at the minimum operable power level of a Raytheon 10 kc, 250 watt sonic oscillator. The suspensions were then diluted and plated with soft agar seeded with *E. coli* B/r. Plaque formation was observed 18 hours later.

Protocol for controls was as above except that the electron beam was off. Additional controls included non-evacuated filter samples and direct inoculations of the seeded agar

with the initial phage suspension. Recovery of phage activity after deposition on filters, evacuation, and sonication was 25 to 50 per cent of the direct controls. The loss was considered caused by possible dehydration, failure of phage to be released from filters, or inactivation caused by surface effects. It was found in initial studies that presoaking the (10 mμ) filters overnight in broth greatly increased recovery. Inactivation by the sonication was considered small since recovery was independent of either sonication time or power over a broad range of values. All fluids used in the studies were prefiltered through 10 mμ to 50 mμ membrane filters to remove extraneous particles.

RESULTS

A previous report (7) presented results obtained with 0.75 to 90 kev electron beam irradiations of T2 bacteriophage using 10 mμ filters. Studies were continued on finer porosity (4 mμ) membrane filters in an attempt to reduce the population fraction which appears protected from low voltage beams. The protection was considered to be caused by shielding, by overlying fluid, of phage particles which settle into filter surface irregularities. Typical survival data for a number of separate experiments are shown in Figs. 2 and 3. A fraction of the particles remained active even for very large values of incident exposure as shown by the "tailing" of Fig. 2.

A summary of the data is given in Table I. The listed cross-sections represent

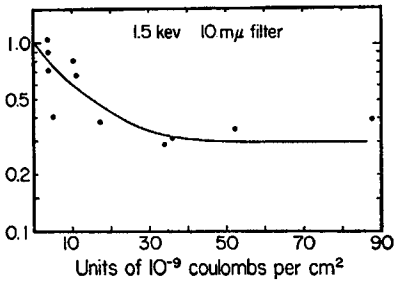

FIGURE 2 Typical survival data for electron beam–irradiated T2 phage. Fractional survival plotted against incident beam charge per unit area. 1.5 kev beam. Phage on 10 mμ porosity filter.

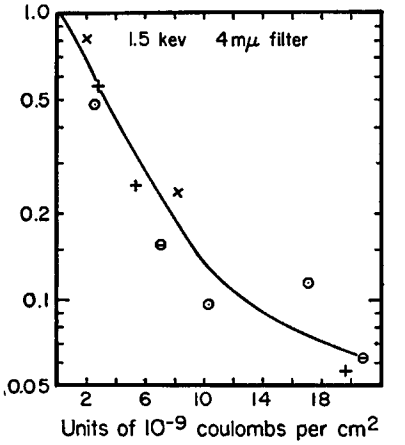

FIGURE 3 Typical survival data for electron beam-irradiated T2 phage. Fractional survival plotted against incident beam charge per unit area. 1.5 kev beam. Phage on 4 mμ porosity filter.

TABLE 1

SUMMARY OF DATA
ELECTRON BEAM IRRADIATIONS OF T2 PHAGE ON BROTH-SATURATED MEMBRANE FILTERS IN 6 mm Hg PRESSURE WATER VAPOR ATMOSPHERE

Initial beam energy	Approximate residue beam penetration	10 mμ filter backing				4 mμ filter backing			
		Cross-section	Resistant fraction	Estimated D_{37} ave.	Estimated D_{37} local	Cross-section	Resistant fraction	Estimated D_{37} ave.	Estimated D_{37} local
kev	*μg/cm²*	*10^{-3} μ²*	*per cent*	*krads*	*krads*	*10^{-3} μ²*	*per cent*	*krads*	*krads*
0.75	1.8	1.07	~50	37	202	0.8	~20	49	270
1.0	4.0	1.14	~45	79	208	4.0	~15	23	59
1.5	8.0	1.5	~30	107	138	4.0	~10	39	50
2.0	13.7	2.3	~15	94	94	5.3	~5	40	40
3.0	29.0	3.2	~10	70	70	4.0	~5	55	55
5.0	72.0	3.6	~5	30	30	—	—	—	—
10.0	235.0	1.8	—	32	32	1.07	—	54	54
20.0	810.0	0.67	—	41	41		—	—	—
90.0	11,300.0	0.19	—	42	42	—	—	—	—

the average area per incident electron for a beam exposure yielding 63 per cent inactivation of the sensitive fraction of the population. The apparent protected or resistant fraction is listed for each beam voltage. Penetration depths and dose calculations were based on studies, with electron beams of 20 ev to 80,000 ev energies, of beam transmission and scattering and ionization and energy transmission and reflection in collodion foils (and air) (8, 9). These studies also provided estimates of the rate of energy loss (dE/dx), and the depth dose, particle flux, and average energy per particle at any penetration depth.

The average D_{37} dose listed in Table I is defined as the energy, at a 37 per cent survival level, deposited per phage divided by the phage mass, while the local D_{37} dose is this energy divided by only the phage mass up to the penetration depth of the beam. Penetration depth is defined as the absorption thickness which transmits 2 per cent of the incident beam energy. The mean path length through the phage was taken to be about 0.08 μ or about equivalent to 11 μg/cm² of collodion.

The apparent protected or resistant fraction was much reduced when a 4 mμ filter was used. The earlier work is felt to be in error at the lower beam energies because of the difficulty of properly correcting for the effects of the large resistant fraction. A plot of the data is given in Fig. 4. An abrupt rise in the cross section between 0.75 and 1.0 kev is followed by a peak value of about 5.3×10^{-3} μ² at 2 kev after which the cross section falls continuously with increasing voltage. The average D_{37} dose appears to attain a minimum value of about 23 kr for a 1 kev incident beam. The indicated values are determined to no better than ± 30 per cent because of scatter in the original data.

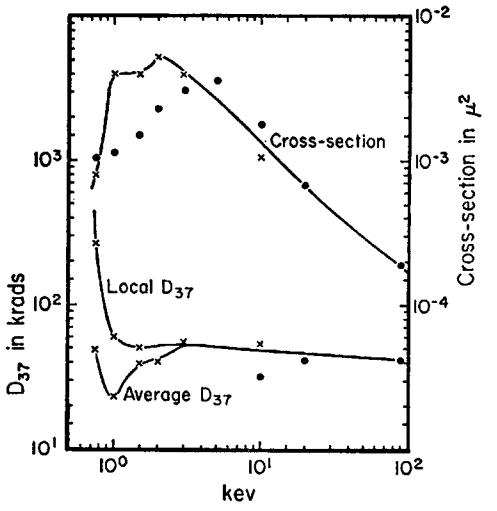

FIGURE 4 Plot of measured electron cross-sections and the estimated local and average doses yielding 37 per cent survival for initial beam energies from 0.75 to 90 kev. Dots refer to 10 mμ filter data and crosses to 4 mμ filter data.

DISCUSSION

Interpretation of the effects of partly penetrating electron beams is complicated by the fact that particle number, energy flux, (dE/dx), and depth dose vary as the beam penetrates an absorber. For example, roughly one-half of the incident particles and three-fourths of the incident energy is absorbed within the first half of the electron range (9). Thus, if a sensitive structure is surrounded by an inert coat, an electron beam penetrating twice the coat thickness would exhibit only one-half the apparent cross-section and one-fourth the efficiency (energy-wise) of a beam that projects most of the particles and energy into the sensitive volume. Thus, the coat thickness is roughly one-half the range of a beam yielding one-half the maximum cross-section. Furthermore, if the internal structure were uniformly sensitive so that the probability of inactivation were simply proportional to the total energy expended within the sensitive region no matter where that energy is placed, then the average D_{37} dose (dose to the whole phage) would decline to a constant value as beam voltage is decreased to the point at which either a small or constant fraction of the beam energy is expended in the inert coat. Correspondingly, the local D_{37} dose (dose to the irradiated region) would decline more or less continuously until the beam completely penetrates the whole particle after which the local dose is equivalent to the average dose. However, if inactivation also depends on the location of energy deposited within a sensitive region, the response may be quite different. For instance, if the inert coat surrounds a sensitive structure which is in the form of a shell surrounding an inert core, the average D_{37} dose would decline with increased beam penetration until a maximum fraction of the incident energy is projected into the top hemisphere of the sensitive shell, after which the D_{37} dose would rise to a

constant value since the fraction of the energy which is projected into the inert core would be ineffective. The local D_{37} dose would tend to decline to a fairly constant value once the sensitive shell is penetrated since this dose will roughly represent the actual dose available in the sensitive structure.

Effects caused by dependence of inactivation probability on dE/dx might further complicate interpretations. dE/dx has been measured in air and collodion for electrons of 20 to 50,000 ev energies (9). A saturation value of about 25 kev/μ was observed below 500 ev. Since the relative biological efficiency of heavy particles changes only slowly (generally less than 50 per cent) for dE/dx values below 25 kev/μ (10), an electron dE/dx dependence likewise may not be large. However, one may need to consider also the amount of energy transferred by an electron to small regions. The latter may be appreciably greater than dE/dx because of scattering. For instance, the initial rate of energy loss per unit *penetration* distance, dE/dt or linear depth dose, was measured in excess of 100 kev/μ for electron energies below 500 ev (9).

In terms of the previous discussion the results of the radiation studies as plotted in Fig. 4 tend to imply that the phage particle contains a relatively radiation-insensitive coat surrounding a radiosensitive shell. The fact that beyond 3 kev the cross-section declines so as to yield a constant D_{37} dose implies that inactivation primarily depends on the radiation energy expended in the sensitive structure (*i.e.*, the particle number and dE/dx are important only so far as they contribute to the energy deposited). For low energy electrons a cross-section equal to one-half the maximum value occurs for a beam of about 900 ev having an initial range of 5 μg/cm^2. The half range of 2.5 μg/cm^2 less the 1.5 μg/cm^2 preabsorption would yield an inert coat thickness of about 1.0 μg/cm^2. However, the low cross-section observed at 750 ev implies an inert thickness comparable to residue penetration of this beam or about 2.0 μg/cm^2. The maximum cross-section observed corresponds to the area expected to be presented by the DNA (85 mμ diameter); hence it appears that the traversal of one 2 kev electron leaves sufficient energy in the sensitive volume to inactivate the phage.

An interesting estimate can be made of the thickness of the radiation-sensitive region by defining

$$t = \frac{V}{S}$$

where t is the apparent inactivation thickness, V is the inactivation volume defined as the volume per average radiation event for a dose yielding 37 per cent survival, where the events are non-overlapping as occurs with low dE/dx electrons and involve an average energy of 60 ev (11, 12), S is the maximum cross-section observed for particles with high dE/dx. The evaluation leads to $t \approx 30$ A.

The physical studies indicate that the linear depth dose, dE/dt, for a 2 kev

electron is in the order of 20 kev per micron in the region of the sensitive structure. Thus, the average energy expended by one incident 2 kev electron within a thickness equal to t is

$$D = t \frac{dE}{dt} \approx 60 \text{ ev}$$

which corresponds to the average energy for a single event and implies that a single event occurring within the rather thin sensitive region leads to phage inactivation.

MODEL OF PHAGE PACKING

If we assume the hydrated phage to be represented approximately by a 1000 A diameter sphere, the protein coat of about 1×10^{-16} gm (13) would constitute an outer layer about 50 A thick. The inner volume of about 3.8×10^{-16} cc would contain about twice the volume represented by the 2×10^{-16} gm of DNA (13). Thus, the internal volume could contain about 50 per cent water content which is approximately the expected hydration (14). If DNA were closely packed in a peripheral shell it would fill a 100 A thick region. The packing, which probably involves a single DNA molecule of 60 μ length (15), should result in a preferential alignment of the DNA fiber along the long axis of the phage (16, 17). Although a simple ordered "ball of string" packing could conform to such conditions, the problem of quickly unwinding such a ball to inject a 60 μ long molecule through the phage tail into a bacterial cell becomes formidable. An alternative packing has been previously proposed (7) which involves an initial coiling of the DNA into a 100 to 150 A diameter fibril about 2.5 μ long which then condenses within the protein coat by an additional level of coiling (Fig. 5). The coilings are suggested to result from molecular dehydration of the DNA and the extension (or injection) to result from molecular hydration.

The results of the radiation studies can be said to correspond to this model in a qualitative way. There are several interesting departures. First, the radiation analysis implies an inert coat thickness of 100 to 200 A which is considerably greater than the 50 A expected of the protein itself. Water of hydration either contained in or on the protein may be responsible for this discrepency. If this is true, it is interesting that diffusion of radiation energy or effect through the hydrated coat to the sensitive structure can not involve dimensions much greater than about 50 A. The second departure is the fact that the suggested packing, although in the form of a shell, contains DNA in a considerable thicker region than is implied for the radiosensitive structure. An explanation may be that the outer surfaces of the DNA packing are more susceptible to radiation damage than are the internal regions possibly because of: (*a*) reversal or restitution of radiation damage which occurs in the internal regions; (*b*) a restraint, imposed by the pack-

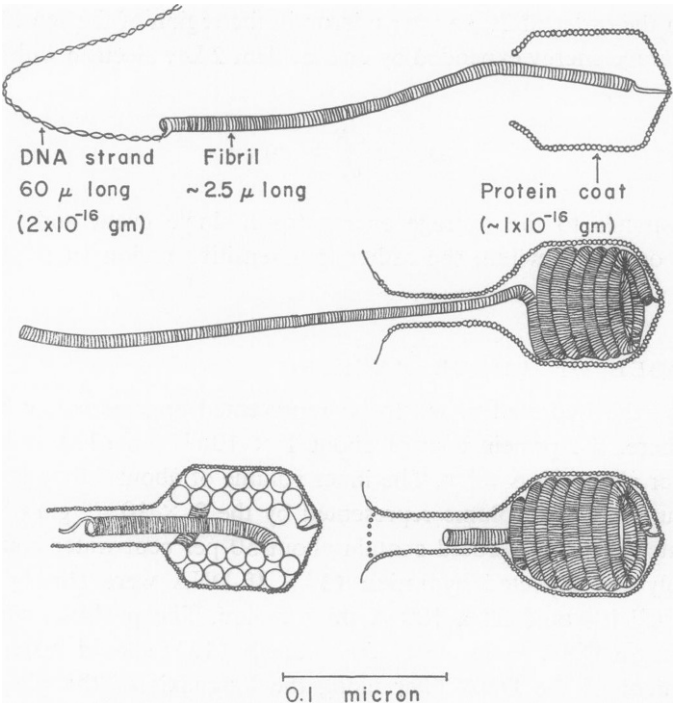

FIGURE 5 Suggested model for DNA packing in T2 bacteriophage.

ing structure, on bond movement or rupture in the internal regions; (*c*) The possibility of cross-linking between the peripheral DNA and protein coat.

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