THE MECHANISM OF INACTIVATION OF T4 BACTERIOPHAGE BY TRITIUM DECAY

FRED FUNK, STANLEY PERSON, *and* RICHARD C. BOCKRATH, JR.

From the Biophysics Department, The Pennsylvania State University, University Park, Pennsylvania 16802. Dr. Funk's present address is the Biology Division, California Institute of Technology, Pasadena, California 91109. Dr. Bockrath's present address is the Department of Microbiology, University of Indiana Medical Center, Indianapolis, Indiana 46204.

ABSTRACT Coliphage T4 was used as a model system to study the mechanism of biological inactivation produced by tritium decay. Experimentally, tritiated precursors were incorporated into phage DNA (thymidine-^{3}H) or into phage protein (^{3}H-amino acids). The ratio of killing efficiencies for decays originating in phage DNA to those originating in phage protein was 2.6. Inactivation by decays from labeled amino acids was assumed to occur exclusively from β-particle irradiation of phage DNA. If decays originating in DNA are due solely to irradiation of DNA, then the killing efficiencies reflect the energy transfer paths in phage DNA for decays originating in phage DNA and in the protein coat. The energy transfer paths were determined for the two cases with the help of a computer and found to be very nearly equal to the experimentally determined ratio (2.6). The killing efficiencies for decays originating in phage DNA were 0.12 and for decays originating in protein 0.046.

INTRODUCTION

In the studies reported here, T4 coliphage is used as a model system to determine the mechanism of biological inactivation produced by tritium decay. Possible events associated with tritium decay which could cause lethality are: (*a*) ionizations occurring along the path of the emitted 0–18 kev β-particle (*b*) transmutation of the parent hydrogen atom to helium, (*c*) absorption by the daughter helium atom of 0–3 ev of nuclear recoil energy and 24 ev of excitation energy, (*d*) molecular rearrangement of the parent molecule occurring as a result of the transmutation from hydrogen to helium. Of these, the ionizations produced by the emitted β-particle and the molecular rearrangement of the parent molecule following decay are the events that are most likely to produce inactivation.

Rachmeler and Pardee (1), Person (2), and Person and Bockrath (3) found that decays originating mainly from thymidine-^{3}H which had been incorporated into the DNA of *E. coli* were two to three times as effective in producing loss of colony-forming ability as were decays from either histidine-^{3}H, leucine-^{3}H, or proline-^{3}H which

had been incorporated into bacterial protein. Person (2) suggested that the relatively high effectiveness of decays of thymidine-^{3}H might indicate the existence of molecular rearrangement at the site of the decay. On the other hand, Koch (4) showed the experimentally determined efficiencies could be explained on the basis of differential β-particle ionization damage within the cell. Approximating the bacterial volume by a sphere, he calculated the relative radiation doses to a central volume of the sphere (of radius one-half that of the sphere) for tritium decays originating in the central volume and for tritium decays originating throughout the sphere. He found considerably higher radiation doses to the central volume for decays originating within the central volume. Thus, if the DNA of a bacterial cell is primarily confined to such a central volume and if the protein is uniformly distributed, the experimentally observed killing efficiencies could be explained by differential β-particle irradiation of the DNA.

A simpler and more defined biological system is needed to further test this conclusion. Therefore T4 bacteriophage was chosen as an alternate model system for a study similar to those just described for bacteria. The dimensions of the whole phage and the distribution of protein and DNA within the phage are better established than they are for bacteria. Phages within a population are quite uniform in size, shape, and DNA content. For autoirradiation by tritium β-particles originating in phage DNA and protein, the DNA is the radiation-sensitive material since the radiation dose to the medium and to the attachment sites will be much less than to the DNA. Finally, since the dimensions of the phage head are small compared to the average range of the tritium beta particle, the rate of energy loss by a β-particle traversing the phage head is nearly constant allowing a more accurate calculation of the relative energy loss in the DNA per decay than can be done for bacteria.

In this study we have determined values of the killing efficiency, α, the probability that a single decay produces loss of plaque-forming ability for decays originating in T4 phage DNA, for example, using thymidine-^{3}H or uracil-6-^{3}H (incorporated as hydroxymethylcytosine-6-^{3}H)[1] as a label, and for decays originating in phage protein using tritiated amino acids as a label. We find decays in DNA to be 2.6 times as effective in causing inactivation of T4 as those originating in phage protein. Inactivation by decays from tritiated amino acids is assumed to occur only from those β-particle ionizations which are produced in the DNA. If inactivation by decays from thymidine-^{3}H or hydroxymethylcytosine-6-^{3}H is due solely to the radiation effect, then the killing efficiencies should reflect the relative energy transfer paths in the phage DNA when decays originate in DNA and in the protein coat. With the help of a computer, the average energy transfer paths through the phage DNA were determined for the two cases. The calculated ratio of paths (DNA/protein) is very nearly equal to the experimentally determined ratio of killing efficiencies (2.6). From

[1] In *E. coli*, uracil and cytosine are rapidly interconverted. If one uses uracil as label, a few minutes after the introduction of the label, one-half of the label is found as uracil and one-half as cytosine. The cytosine is then converted to hydroxymethylcytosine in T4 infected cells.

this agreement we conclude that no molecular rearrangement of the parent molecule that is important for inactivation of T4 occurs from decays of thymidine-^{3}H or hydroxymethylcytosine-6-^{3}H originating in phage DNA.

MATERIALS AND METHODS

Stock Cultures and Media

The phages used were T4 (thy-) and T1. The former is a thymine-requiring strain obtained from Dr. I. Tessman (Purdue University, Lafayette, Ind.) who isolated it from a T4 BO_1 parent strain (5). T1 was obtained from Dr. E. Pollard of this department.

E. coli B-3, a thymine-requiring strain of *E. coli* B obtained from Dr. N. Symonds (University of Sussex, Sussex, England), was used as the host for labeling phage. Assays for plaque-forming ability utilized Hershey's strain of *E. coli* S which was obtained from Dr. J. Cairns (Carnegie Institution of Washington, Cold Spring Harbor, New York).

Two growth media were used to label phage. The first medium, phage A-1, in which nearly all thymine-^{3}H and ^{3}H-amino acid labelings were carried out, contained 2 g NH_4Cl, 3 g Na_2HPO_4, 1.5 g KH_2PO_4, 4 g NaCl, 115 mg Na_2SO_4, 34 mg $MgCl_2 \cdot 6H_2O$, 100 mg gelatin, 8 g glucose, and 100 mg tryptophan per liter of distilled water. The second medium in which all uracil-6-^{3}H and a few thymidine-^{3}H and ^{3}H-amino acid labelings were carried out contained 5.8 g NaCl, 3.7 g KCl, 100 mg $MgCl_2 \cdot 6H_2O$, 1.1 g NH_4Cl, 142 mg Na_2SO_4, 272 mg KH_2PO_4, 10 ml 50% glucose (w/v), 12.1 g Tris, and 15 g vitamin-free casamino acids per liter of distilled water. The final medium was adjusted to pH 7.5 with HCl. Casamino acids were omitted for labeling with tritiated amino acids.

The bottom agar in plates for assay of plaque-forming ability contained 6 g tryptone, 16 g yeast extract, 5.8 g NaCl, and 15 g of agar per liter of distilled water. Top agar contained 8 g nutrient broth, 5.8 g NaCl, and 4.5 g of agar per liter of distilled water. When plating T1, 8.0 g of top agar was used. Host cells were grown in nutrient broth (8 g/liter). Phage were generally diluted in nutrient broth before assaying for loss of plaque-forming ability.

Method of Labeling and Purification

The specific activities and concentrations of the tritium compounds used are given in Table I. All tritium compounds were evaporated to dryness in the phage growth tubes before use. Log phase host cells at 2–3 $\times$ 10^8/ml were filtered and washed before being added to the phage growth tube. In addition, when labeling with tritiated amino acids, the host cells were glucose-starved in phage A-1 medium less glucose, tryptophan, and gelatin for 30 min before phage infection to increase the specific activity of labeled phage. Glucose, tryptophan, and gelatin were added at the time of phage infection to make the normal final concentrations.

Phage were added to 1.0 ml of cells at a multiplicity of infection of 3–4 and incubated with aeration for about 45 min. Chloroform was added to the culture to enhance lysis, and lysates were clarified by two low-speed centrifugations (7500 rpm, 10 min, Servall superspeed centrifuge, Ivan Sorvall, Inc., Norwalk, Conn.). For thymine-^{3}H labeled phage, the lysate was then treated with 50 μg/ml of DNAse for 30 min at 37°C. For hydroxymethylcytosine-6-^{3}H labeled phage, the lysates were also treated with RNAse at 50 μg/ml and ethylenediaminetetraacetate (EDTA) at 5 $\times$ 10^{-3} M for 20 min following the DNAse treatment.

Radioactive phage were further purified by one of two methods. In the first method the lysates were dialyzed for 48–72 hr with changes in the dialysis fluid every 12 hr. Dialysis fluid contained 0.1 M NaCl, 0.001 M $MgCl_2$, 0.01% gelatin, and 0.01 M Tris buffer, adjusted to

TABLE I

SPECIFIC ACTIVITIES AND FINAL CONCENTRATIONS OF THE TRITIUM ISOTOPES USED

Compound	Final concentration	Specific activity	Source
	$\mu g/ml$	Ci/mM	
Thymine-^{3}H*	4	15.7	New England Nuclear Corp. (Boston, Mass.)
Thymidine-^{3}H*	8	17.4	New England Nuclear Corp.
Uracil-6-^{3}H‡	10	10.0	New England Nuclear Corp.
Leucine-^{3}H	8	5.5	New England Nuclear Corp.
Lysine-^{3}H	8	4.1	New England Nuclear Corp.
Proline-^{3}H	8	5.0	New England Nuclear Corp.
Phenylalanine-^{3}H	8	1.65	Schwarz Bio Research Inc. (Orangeburg, N. Y.)
Histidine-^{3}H	8	1.1	Schwarz Bio Research Inc.

* For thymine-^{3}H and thymidine-^{3}H the tritium atom is located on the methyl group.

‡ For uracil-6-^{3}H the tritium is located on the six position of the pyrimidine ring.

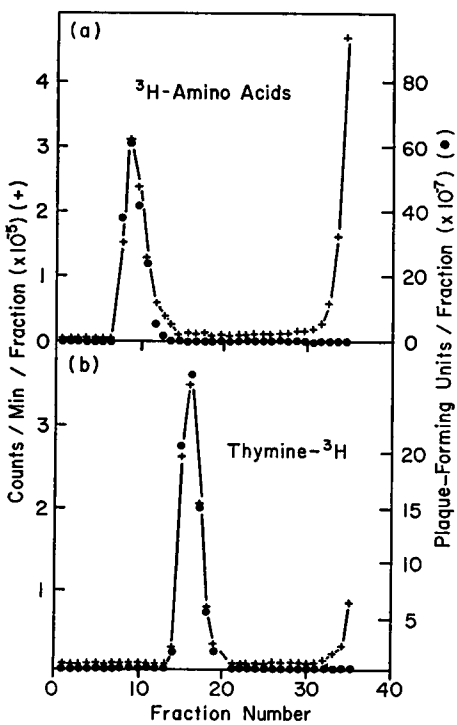

FIGURE 1 Cesium chloride density gradient centrifugation of dialyzed lysates of T4 labeled with thymine-^{3}H and tritiated amino acids. Recovery of radioactivity (+) and biological activity (○) are plotted as a function of the fraction number.

pH 7.5, per liter of distilled water. Dialysis was followed by two low-speed centrifugations and banding in a cesium chloride gradient (Spinco Model L, SW39L head (Beckman Instruments, Inc., Palo Alto, Calif.), 35,000 rpm, 30 hr, 7°C). Samples of the fractions were collected and assayed for biological activity and radioactivity. Fractions from the cesium chloride

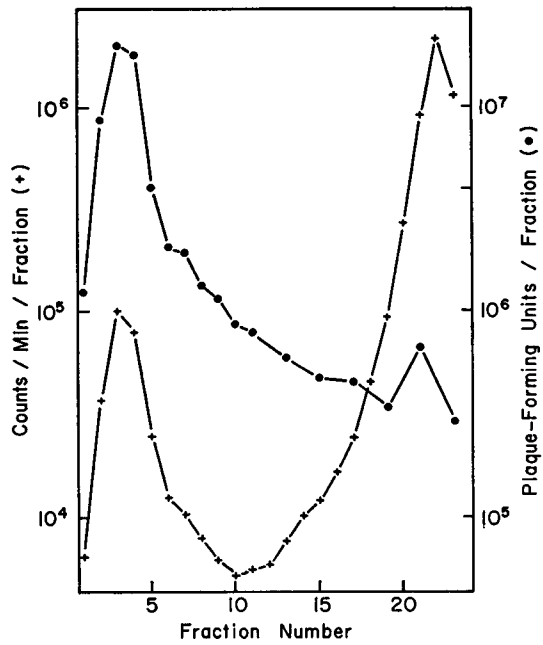

FIGURE 2 Sucrose gradient centrifugation of T4 labeled with uracil-6-^{3}H (incorporated as hydroxymethylcytosine-6-^{3}H). The radioactivity (+) and biological activity (○) recovered are plotted on a logarithmic scale as a function of the fraction number. Direction of sedimentation is to the left.

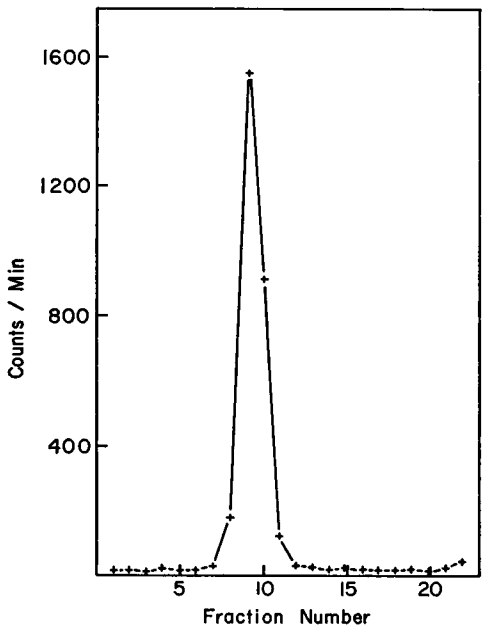

FIGURE 3 Cesium chloride density gradient centrifugation of fractions 3 and 4 of Fig. 2. The recovery of the radioactivity is plotted as a function of the fraction number.

were never subjected to more than 1.0 M changes in salt concentration to avoid osmotic shock. The biological activity and radioactivity profiles for a T4 lysate labeled with the five tritiated amino acids are shown in Fig. 1*a*. A significant amount of the radioactivity recovered occurs

at the top of the tube. This result is typical of lysates labeled with tritiated amino acids and may represent phage tails or empty heads which would band at this position. The contaminating radioactivity, however, is well separated from the phage band that contains 90–95% of the biological activity. Profiles for the T4 lysate labeled with thymine-^{3}H are shown in Fig. 1*b*. Nearly all the radioactivity is associated with the biological activity in the phage band. The difference in the positions of the phage bands in Fig. 1*a* and 1*b* is due to a difference in the beginning density of cesium chloride and not to a difference in phage density.

In the second method of purification, successive sucrose gradient and cesium chloride density gradient centrifugations were used. After the nuclease treatment, 0.05 ml of the lysate was sedimented in a 5–20% sucrose gradient (SW39L head, 31,500 rpm, 11 min, 7°C). Sucrose was dissolved in water containing 0.1 M NaCl, 0.01 M Tris, and 0.001 M $MgSO_4$ at pH 7.5. Fractions were collected in the same solution without sucrose. The biological activity and radioactivity profiles obtained following sucrose gradient sedimentation are shown in Fig. 2. The particular example shown is T4 labeled with uracil-6-^{3}H (incorporated as hydroxymethylcytosine-6-^{3}H). The radioactivity profile of Fig. 2 shows a large amount of radioactivity from unincorporated tritiated precursors at the top of the centrifuge tube because the lysates were not dialyzed. Two or three fractions from the phage band were pooled and a portion of this was rebanded in a cesium chloride gradient for a further check of purity. For this particular lysate, equal volumes of fractions three and four representing 60% of the biological activity recovered were used. 95% of the radioactivity recovered from the cesium chloride gradient (as plotted in Fig. 3) is associated with the main radioactive band.

Determination of the Radioactivity Incorporated per Phage

λN^* denotes the specific activity of the phage in disintegrations per phage per unit time. λ is the probability that a tritium atom will decay per unit of time and N^* is the number of tritium atoms per phage. Values for λN^* were obtained by dividing the number of disintegrations occurring per unit time in a given volume by the number of phage in that volume. The phage concentration was determined by extrapolating the survival curves to zero decays per phage and assuming a plating efficiency of one. The radioactivity determinations were made on fractions from the phage band of the cesium chloride (purification method one, Fig. 1) or sucrose (purification method two, Fig. 2) gradients. We assume a negligible loss of phage particles occurred during purification. Samples were put on filter paper (1 cm^2) and dried. The filter paper was combusted in a special flask in an oxygen atmosphere that reduces the tritiated compounds to tritiated water (6). This was condensed by cooling and recovered by adding liquid scintillation fluid, returning to room temperature, and shaking to dissolve the water. In order to correct for small losses and/or quenching due to combustion, an aliquot of uracil-^{3}H (containing cesium chloride where this was present) was treated in a similar way and the amount of radioactivity recovered compared with that for an equal aliquot pipetted directly into scintillation fluid. Combustion was used because of the reduced counting efficiency when samples of phage suspended in cesium chloride were introduced directly into scintillation fluid. This was probably due to precipitation of the phage by the cesium chloride. Determinations of radioactivity were made in a naphthalene-dioxane base scintillation fluid in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The counter efficiency (15–18%) was determined by using a tritium water standard (National Bureau of Standards, Washington, D. C.).

Storage of Lysates

Phage used for survival curves were removed from the lysate immediately after nuclease treatment, and also after dialysis, and diluted 1:100 into either phage A-1 less glucose, tryptophan, and gelatin or 0.8% nutrient broth and stored at 5°C. Plaque-forming ability was followed over a period of 2–6 wk depending upon the tritiated precursor used. Survival curves from dialyzed and undialyzed lysates had the same slope indicating that no inactivation occurs from decays of unincorporated tritium present in the medium.

Lysates of unlabeled phage used as a control for each experiment were usually stable. In a few of the experiments carried out over longer periods of time (3–6 wk), such as the inactivation of T4 with ^{3}H-amino acids, declines in the biological activity of controls to as much as 50% survival were observed. Where this occurred, the tritium survival curve was corrected for the control decline.

RESULTS

Survival Curves

Representative survival curves are plotted in Figs. 4 and 5. The fraction of phage surviving (N/N_0) is plotted against the accumulated number of decays per phage ($\lambda N^* t$). The curves are described by the relation

$$N/N_0 = e^{-\alpha \lambda N^* t}.$$

N/N_0 represents the fraction of phage surviving after time t, λN^* represents the rate of accumulation of decays per unit time, and α is the probability that a single decay will inactivate.

Inactivation from accumulated decays of thymine-^{3}H and ^{3}H-amino acids in T4 are

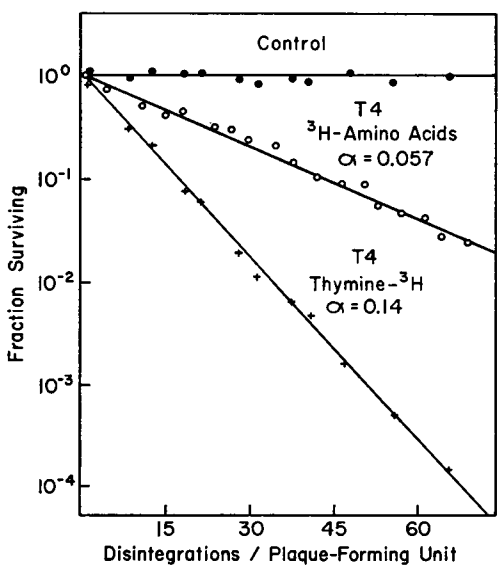

FIGURE 4 Inactivation of T4 from decays of thymine-^{3}H and tritiated amino acids. The fraction surviving is plotted on a logarithmic scale as a function of the average number of accumulated disintegrations per plaque-forming unit. The survival of the control for the thymine-^{3}H labeled culture is indicated at the top of the figure. The killing efficiency, α, the probability that a single decay will inactivate, was 0.057 for decays of ^{3}H-amino acids and 0.14 for decays of thymine-^{3}H. Phage were stored at 5°C to accumulate decays.

shown in Fig. 4. For the data shown, decays from incorporated thymine-^{3}H are about 2.5 times as effective in causing inactivation as decays from incorporated ^{3}H-amino acids. The inactivation of T4 from decays of hydroxymethylcytosine-6-^{3}H is shown in Fig. 5. Decays of thymine-^{3}H or hydroxymethylcytosine-6-^{3}H are equally effective in producing lethality in T4. In order to be certain that the label from DNA, precursors, thymine-^{3}H and uracil (incorporated as hydroxymethylcytosine-6-^{3}H) was being incorporated into the DNA of the phage, and that label from the ^{3}H-amino acids was going into the phage protein, samples of purified lysates labeled with each of the three tritiated precursors were phenol-extracted. The extraction procedure was essentially that of Guthrie and Sinsheimer (7). For phage labeled with

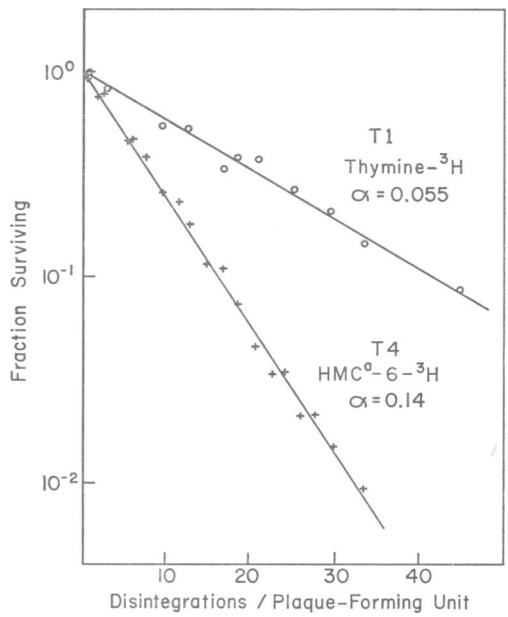

FIGURE 5 Inactivation of T1 from decays of thymine-^{3}H, and inactivation of T4 from decays of hydroxymethylcytosine-6-^{3}H. The fraction surviving is plotted on a logarithmic scale as a function of the average number of accumulated decays per plaque-forming unit. The killing efficiency, α, was 0.055 for decays of thymine-^{3}H in T1 and 0.14 for decays of hydroxymethylcytosine-6-^{3}H in T4. [a] Uracil-6-^{3}H, the precursor actually used, is converted to HMC-6-^{3}H hydroxymethylcytosine-6-^{3}H) in the infected cell.

thymine-^{3}H or uracil-6-^{3}H, more than 96% of the label was found in the aqueous phases of the first and second extractions, indicating that the tritium had been incorporated into the DNA. Similarly for T4 labeled with ^{3}H-amino acids, 98% of the label occurred in the phenol or protein phase.

The inactivation of T1 by decays of thymine-^{3}H is also shown in Fig. 5, and as can be seen from the figure, they are less effective in producing inactivation than the corresponding decays in T4. Survival curves for T1 labeled with thymine-^{3}H stored in phage A-1 less glucose, tryptophan, and gelatin had a slightly greater slope than those obtained for storage in 0.8% nutrient broth. The protective effect of the nutrient broth is not large but was observed for all four experiments done. A medium effect, for these two storage media, was not observed in the case of T4 inactivation.

A tabulation of all the values of α obtained for T4 and T1 is given in Table II. The average values of α for decays of thymine-^{3}H and hydroxymethylcytosine-6-^{3}H in T4 are both 0.12. The average value of α for decays of ^{3}H-amino acids in T4, however, is only 0.046, less than half the value obtained for decays occurring in the DNA of the phage. The average values of α obtained for decays of thymine-^{3}H in T1 are 0.051 for phage stored in broth and 0.069 for phage stored in minimal medium. The determination of a particular α assumes a plating efficiency of one, but to obtain relative killing efficiencies we need only assume that the plating efficiency does not vary from experiment to experiment.

TABLE II

TABULATION OF KILLING EFFICIENCIES FOR T4 AND T1 BY TRITIUM DECAY

T4			T1	
Thymine-^{3}H* or Thymidine-^{3}H	HMC-6-^{3}H‡	^{3}H-amino acids	Thymine-^{3}H	Thymine-^{3}H
0.079	0.084	0.057	0.055	0.087
0.12	0.089	0.056	0.046	0.074
0.15	0.13	0.043	0.043	0.045
0.14	0.095	0.033	0.061	0.071
0.11	0.12	0.039		
0.14	0.16			
0.14	0.16			
0.11	0.14			
0.12				
0.12				
0.12	0.12	0.046	0.051	0.069

* Values of α listed are for thymine-^{3}H or thymidine-^{3}H.
‡Hydroxymethylcytosine-6-^{3}H.

The values of α obtained for T4 from decays of thymine-^{3}H, uracil-6-^{3}H (hydroxymethylcytosine-6-^{3}H), and ^{3}H-amino acids are listed in columns 1, 2, and 3. The values of α obtained for T1 from decays of thymine-^{3}H are listed in columns 4 (storage in 0.8% nutrient broth) and 5 (storage in phage A-1 less glucose, tryptophan, and gelatin). The average value of α obtained for a particular isotope is given in the last row of each column. All killing efficiencies were determined at 5°C.

The Computer Program

The computer program was designed to simulate tritium decays in a geometrical volume of proportions matching the T4 phage head. The phage head volume was approximated by a cylinder 800 A in diameter, 300 A long, having hemispherical ends 800 A in diameter giving a phage of dimensions 800 × 1100 A (8, 9). The DNA volume was designed to contain a void in its center 70 A in diameter (10). A void of

15 A was also placed between the DNA volume and the protein coat to agree with the observations of Dr. T. Anderson.[2] The computer cycled through 3000 simulated decays in the following manner. A point within the phage protein (protein-labeled) or DNA (DNA-labeled) volume was chosen randomly. A straight-line path was then stepped off in a random direction. The step size was 25 A and sufficient steps were taken to traverse the phage in any manner. The decrease in energy transfer per step was taken from a representative energy distribution for tritium β-particles as described in the accompanying paper.[3] A linear decrease in energy deposition of 0.4% per step was used. After each step the computer noted whether the end of the path was in the DNA volume, protein volume, or outside the phage and assigned the

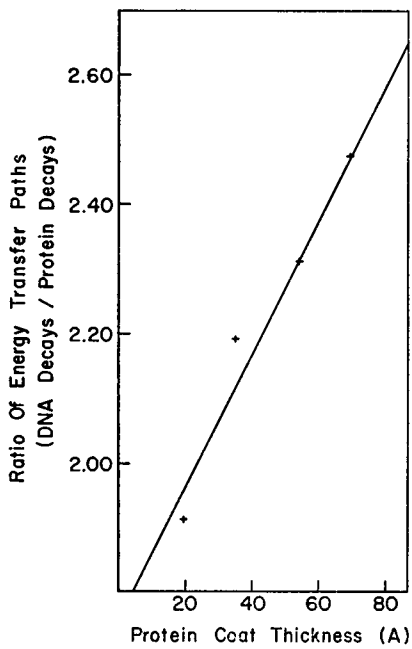

FIGURE 6 The ratio of the energy transfer paths through the DNA of T4 from decays originating in the DNA to those originating in protein is plotted as a function of the protein coat thickness. The line drawn through the points was taken from smooth curves drawn through the data points of individual plots of energy transfer paths vs. protein thicknesses for DNA and protein decays. The points in Fig. 6 represent the actual ratio of the data points from these plots. The difference between the points and the line is small and is due to the limited number of decays (3000) simulated in the computer for each protein thickness.

energy deposition for that step accordingly. The length of the DNA energy transfer path for one simulated decay was determined by summing the energy depositions for all steps which ended within the DNA volume. The effective DNA energy transfer path was determined by averaging over 3000 decays.

Effective DNA energy transfer paths were calculated for decays originating in DNA and phage coat protein for a number of protein coat thicknesses. The ratio of calculated paths (for DNA/protein decays) for T4 is plotted in Fig. 6 as a function of protein coat thickness.

[2] Personal communication.
[3] R. C. Bockrath, Jr., S. Person, and F. Funk. Companion article.

Protein Membrane Thickness

Rather large variations in measurements of the protein coat thickness of T-even phage can be found in the literature. Cole and Langley (11) find a coat thickness of 100–200 A as measured by the differential penetration of low energy electrons. Favre et al. (8) measured the thickness of a flattened polyhead of T4 from electron micrographs to be 100–120 A making the single membrane thickness 50–60 A Polyheads, however, are made by mutant phage and hence may not be representative of the actual T4 protein coat. They also estimate the coat thickness of T4 from differences in widths measured from whole phage and from sections showing only the DNA volume. This difference is about 100 A, corresponding to 50 A for the coat thickness. Cota-Robles and Coffman (12) measure a membrane thickness of 90 A. Measurements by Anderson[2] indicate that, for thin sections of whole phage, the coat thickness might be as low as 35 A including a 15 A void between the protein and DNA. Values of 35 A were found for thin sections of phage ghosts. Brenner et al. (13) also measure a protein thickness of 35 A for ghosts. However, they indicate that one would predict a thickness of 60 A from the molecular weight of the head protein. Finally, measurements by Klimenko, Tikckonenko, and Andreev (14) (from electron micrographs of Bradley) were from 35–70 A depending on the method of preparation. We conclude that the protein coat thickness lies in the range of 35–90 A.

Correction for Internal Protein and Tail Protein

A lower limit for the coat thickness must be about 35 A. A value of 35 A for the coat thickness corresponds to a ratio of energy transfer paths from Fig. 6 of 2.12. This ratio must be corrected for those decays that occur in internal protein and tail protein since these were not programmed into the computer. β-particles originating in internal protein, 7 % of total protein (15), will have the same energy transfer paths as decays originating in DNA, assuming the internal protein is uniformly distributed through the DNA volume. Those originating in tail protein, about 11 % of total protein, assuming a total protein molecular weight of 10^8 (13, 16, 17), will have DNA energy transfer paths much smaller than decays originating in coat protein. From solid angle considerations we have concluded that these β-particles will have DNA energy transfer paths that are 0.14 of those originating in coat protein. Consideration was also given to specific activity of the coat, internal protein, sheaths, and tail core due to the different amino acid compositions of each (15, 17, 18). For example, the amino acid compositions indicate that the labeled amino acids used are twice as prevalent in the sheath and core protein as in the coat. The total correction results in an 8 % increase in the ratio of pathlengths giving a corrected ratio of 2.28.

DISCUSSION

The average values of α for T4 obtained for decays of thymine-^{3}H and hydroxymethylcytosine-6-^{3}H were both 0.12. Hence, we conclude that decays of either pre-

cursor incorporated into T4 DNA probably cause inactivation by the same mechanism. The average value of α for decays of the five tritiated amino acids was 0.046. The ratio of the killing efficiencies for decays originating in phage DNA to that for decays originating in phage protein is $0.12/0.046 = 2.6$. The corrected ratio of energy transfer paths for the minimum protein coat thickness considered (35 A) was 2.28. This ratio is in good agreement with that found for killing efficiencies for DNA decays to protein decays of 2.6. Larger values of the protein coat thickness would of course increase the ratio of effective pathlengths. For example, a protein coat thickness of 70 A would give a corrected ratio of pathlengths of 2.66. Decays originating predominantly in head protein are assumed to inactivate solely by β-particles causing ionizations as they pass through the phage DNA volume. If decays originating in the DNA volume cause appreciable inactivation by a molecular rearrangement of the parent molecule, then the experimental ratio (2.6) should be appreciably greater than the calculated ratio ($\geqq 2.3$) and it is not. From this agreement we conclude that only the primary ionizations produced by the tritium β-particle need be considered to explain the inactivation of T4 from decays of incorporated thymine-^{3}H or hydroxymethylcytosine-6-^{3}H. This is the same conclusion reached by others (4; see also footnote 3) for inactivation by tritium decay in bacteria.

A value of the killing efficiency from decays of ^{3}H-amino acids was not obtained for phage T1 and hence we have no internal standard for decays not within the radiation-sensitive material. The killing efficiency obtained, however, is approximately what one would expect from the relative β-particle pathlengths in T4 and T1 DNA and the inherent radiosensitivity of the DNA of T4 and T1 (19).

Two previous survival curves exist in the literature from which one can calculate values of α for decays of thymine-^{3}H in T-even phage. Both calculations of α must be made using a calculated value of λN^* from a knowledge of the DNA content of the phage and the specific activity of the medium since measured values of λN^* of the phage were not reported. For the data of Cairns (20) for T2, one must also account for the dilution in specific activity of the medium by the unlabeled thymine available to the phage from the degraded host DNA. Making this correction and using a DNA molecular weight of 130×10^6 daltons, we calculate an α of 0.12. Making similar assumptions for T4, we have also calculated a value of α from Caro's data (21) for thymidine-^{3}H decays and find an α of 0.12. The values from Cairns' data for T2, 0.12, and Caro's data for T4, 0.12, both agree very well with the value of α we obtain for thymine-^{3}H and hydroxymethylcytosine-6-^{3}H decays in T4. No variation would be expected between our value of α for ^{3}H decays in T4 and that of Caro's due to the difference in storage medium since we find no difference in the rate of inactivation when phage are stored in minimal media or broth. We do not understand why such a difference, even though it is small, exists for T1 and not for T4. No experiments were done using cytosine-5-^{3}H, for which localized damage has

been shown to occur (22) causing C → T transitions (23, footnote 4). If one attempts to label T4 with cytosine-5-^{3}H, the label will be removed from the original precursor by the phage in the process of synthesizing hydroxymethylcytosine.

Using the measured values of α, the effective β-particle DNA pathlengths for decays of thymidine-^{3}H and hydroxymethylcytosine-6-^{3}H, and the value for the initial energy transfer for a representative tritium β-particle, the absolute radiation doses to the DNA of T4 were calculated. The value of the energy transfer per 25 A for a representative tritium beta particle was taken from an extrapolation of the representative energy distribution of Bockrath, Person, and Funk[3] and was 3.78×10^{-11} ergs/25 A. The product of this value and the effective energy transfer path is a measure of energy deposition/decay. This was converted to rads/D_{37} using a hydrated weight of the DNA (230×10^6 daltons) and the number of decays/D_{37}. The calculated dose was 83.5 krads per D_{37}. Using a value of $\alpha = 0.10$ for thymine-^{3}H decays in T2, a spherical approximation of T2, and a rate of energy loss calculated from the Bethe-Bloch equation, Apelgot and Duquesne (24) calculate a D_{37} of 57.6 krads. Using our value of $\alpha = 0.12$, their calculated dose becomes 47.6 krads.

The calculated D_{37} dose of 83.5 krads can be compared with those obtained experimentally, but it is difficult to specify what set of conditions for X-ray inactivation are equivalent to those for inactivation by tritium decays. We suspect that the indirect effects of the beta particle ionizations would be much less important than in X-ray inactivation, and that one should compare the tritium D_{37} radiation dose to X-ray doses obtained under protective conditions. A D_{37} X-ray dose of 40 krads is reported (25) for T4 in nutrient broth at 4°C, and 100 krads under conditions that maximally depress the indirect effect (19). Hence the calculated value for inactivation by tritium decay is in the range expected if the inactivation occurs by β-particle ionizations in the volume of the phage occupied by the DNA.

Dr. Funk is a PHS predoctoral trainee and Dr. Bockrath a predoctoral fellow.
This research was supported by grants NsG-324 from the National Aeronautics and Space Administration and by GB-4485 from the National Science Foundation. We acknowledge the discussions with other workers in our laboratory as well as the technical assistance of Miss Cora Jean Schoenberger and Mrs. Helen Newton.

Received for publication 27 April 1968 and in revised form 7 June 1968.

REFERENCES

1. RACHMELER, M., and A. B. PARDEE. 1963. *Biochim. Biophys. Acta.* **68**:62.
2. PERSON, S. 1963. *Biophys. J.* **3**:183.
3. PERSON, S., and R. C. BOCKRATH, JR. 1964. *Biophys. J.* **4**:355.
4. KOCH, A. L. 1965. *Radiation Res.* **24**:398.
5. SIMON, E., and I. TESSMAN. 1963. *Proc. Natl. Acad. Sci. U.S.* **50**:526.
6. BUYSKE, D., R. KELLY, J. FLORINI, S. GORDON, and E. PEETS. 1963. *In* Advances in Tracer Methodology. S. Rothchild, editor. Plenum Press, New York. **1**:185.

[4] F. Funk and S. Person. Article in preparation.

7. GUTHRIE, G., and R. SINSHEIMER. 1963. *Biochim. Biophys. Acta.* **72**:290.
8. FAVRE, R., E. BOY DE LA TOUR, N. SEGRÉ, and E. KELLENBERGER. 1965. *J. Ultrastruct. Res.* **13**:318.
9. KELLENBERGER, E., A. BOLLE, E. BOY DE LA TOUR, R. EPSTEIN, N. FRANKLIN, N. JERNE, A. REALE-SCAFATI, J. SICHAUD, I. BENDET, D. GOLDSTEIN, and M. LAUFFER. 1965. *Virology* **26**:419.
10. CUMMINGS, D., and T. WANKO. 1963. *J. Mol. Biol.* **7**:658.
11. COLE, A., and R. LANGLEY. 1963. *Biophys. J.* **3**:189.
12. COTA-ROBLES, E., and M. COFFMAN. 1964. *J. Ultrastruct. Res.* **10**:304.
13. BRENNER, S., G. STREISINGER, R. HORNE, S. CHAMPE, L. BARNETT, S. BENZER, and M. REES. 1959. *J. Mol. Biol.* **1**:281.
14. KLIMENKO, S., T. TIKCKONENKO, and V. ANDREEV. 1967. *J. Mol. Biol.* **23**:523.
15. MINIGAWA, T. 1961. *Virology.* **13**:515.
16. SARKAR, N., S. SARKAR, and L. KOZLOFF. 1964. *Biochemistry.* **3**:511.
17. SARKAR, S., N. SARKAR, and L. KOZLOFF. 1964. *Biochemistry.* **3**:517.
18. FITCH, W., and M. SUSSMAN. 1965. *Virology.* **26**:754.
19. GINOZA, W. 1968. *In* Methods of Virology. K. Maramorosch and H. Koprowski, editors. Academic Press, Inc., New York. **4**:139.
20. CAIRNS, J. 1961. *J. Mol. Biol.* **3**:756.
21. CARO, L. 1965. *Virology.* **25**:226.
22. PERSON, S., and R. BOCKRATH, JR. 1965. *J. Mol. Biol.* **13**:600.
23. OSBORN, M., S. PERSON, S. PHILLIPS, and F. FUNK. 1967. *J. Mol. Biol.* **26**:437.
24. APELGOT, S., and M. DUQUESNE. 1964. *Intern. J. Radiation Biol.* **7**:65.
25. WATSON, J. 1950. *J. Bacteriol.* **60**:697.