INACTIVATION OF BACTERIOPHAGES BY DECAY OF INCORPORATED RADIOACTIVE PHOSPHORUS*

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(Received for publication, September 29, 1954)

It was observed by Hershey, Kamen, Kennedy, and Gest (1951) that bacteriophages are unstable if they contain radiophosphorus P^{32} of high specific activity. From day to day, progressively decreasing fractions of such populations of radioactive phage are still able to form plaques when plated on a sensitive bacterial strain, and the rate of loss of infective titer depends on the specific activity of the P^{32} assimilated. It is the purpose of this communication to present experiments in which these observations of Hershey *et al.* have been extended to the study of the lethal effects of P^{32} decay in various strains of bacteriophage at various temperatures and to the examination of some of the biological properties of the inactivated bacteriophage particles. Some of these experiments have already been reported in preliminary form (Stent, 1953 *a*).

Materials and Methods

Bacteriophages T1, T2, T3, T5, T7, and their host, *E. coli* B/r, and phage λ and its host, *E. coli* strain K12S, were used in this study. Strain B/r, a radiation-resistant mutant derived from strain B, was kindly supplied to us by Dr. Aaron Novick.

Glycerol-casamino acid medium refers to a medium devised by Fraser and Jerrel (1953). *H medium* is a glycerol-lactate medium of the following composition per liter of distilled water: 1.5 gm. KCl, 5 gm. NaCl, 1 gm. NH_4Cl, 0.25 gm. $MgSO_4 \cdot 7H_2O$, 10^{-4} N $CaCl_2$, 0.07 M sodium lactate, 2 gm. glycerol, 0.5 gm. bacto-peptone Difco and 0.5 gm. bacto-casamino acids Difco. H medium contains 6 mg./liter total phosphorus, of which 5 mg./liter are supplied by the casamino acids and 1 mg./liter by the peptone. Control experiments show that this phosphorus is assimilated by cultures of *E. coli* neither more nor less readily than inorganic phosphate.

The techniques described by Adams (1950) were employed for the general procedures of bacteriophagy.

Radiophosphorus was obtained as carrier-free $H_3P^{32}O_4$ from the Isotope Division of the Atomic Energy Research Establishment, Harwell, England. Measurements

* This investigation was supported by grants from the National Cancer Institute of the National Institutes of Health, Public Health Service and The Rockefeller Foundation.

‡ Holder of a National Research Council of Canada Special Scholarship.

of radioactivity were made on dry samples by means of an end-window GM tube, whose counting efficiency for P^{32} had been established by reference to a standard solution of radiophosphorus supplied by the National Bureau of Standards, United States Department of Commerce. The specific radioactivity of the growth media was determined by radioactive counting and chemical analysis of total phosphorus in the case of a number of T2 lysates in order to establish the specific inactivation rate αN for that phage and to confirm the value obtained by Hershey *et al.* To conserve the supply of isotope, the specific activity of the growth medium in the case of the other phages was usually estimated only by reference to the rate of inactivation of a stock of T2 grown in an aliquot of the same medium.

Bacteriophages of high specific activity were grown in the following way: A volume of the radioactive stock solution containing the desired amount of P^{32} was evaporated to dryness in a boiling water bath and resuspended in 0.1 ml. of H medium. The radioactive growth medium was then adjusted to neutral pH and inoculated with 0.01 ml. of a culture of 2×10^7 cells/ml. of B/r already in its exponential phase of growth in non-radioactive H medium. The growth of the radioactive culture at 37°C. was followed by microscopic counts in a Petroff-Hausser bacterial counting chamber. When the bacterial density reached 5×10^7 cells/ml., the culture was infected with 0.01 ml. of a stock containing 10^7 phages/ml. and incubated until microscopic counts indicated satisfactory lysis. At this point, the remainder of the 0.1 ml. culture was diluted into cold glycerol–casamino acid medium and assayed for its titer of infective phage particles.

Experimental Results

Rate of Inactivation.—

Hershey *et al.* observed that if a stock of T2 or T4 containing P^{32} at high specific activity was assayed daily, the logarithm of the number of surviving phages fell linearly with the number of P^{32} atoms that had decayed up to the time of assay. The slope of this survival curve was found to be proportional to the specific activity of the medium in which the phages had been grown, provided that the stock was stored in sufficiently great dilution under conditions in which control lysates containing an equal amount of non-incorporated P^{32} were stable. This indicated that the inactivation of one phage particle was not due to the radiation emitted by the radioactivity contained in other phages but was the consequence of the disintegration of one of its own atoms of P^{32}. The rate of change in the fraction s of surviving phage particles with the time t in days may, therefore, be expressed as

$$ds/dt = -\alpha N^* \lambda s \tag{1}$$

in which α is the fraction of the P^{32} disintegrations which are lethal (hereafter referred to as the "efficiency of killing"), N^* the number of radioactive phosphorus atoms per phage particle, and λ the fractional decay of P^{32} per day. Integration of (1) and substitution of more practical parameters lead to

$$\log_{10} s = -1.48 \times 10^{-6} \alpha A_0 N (1 - e^{-\lambda t}) \tag{2}$$

in which A_0 is the specific radioactivity (in millicuries per milligram of phosphorus) of the growth medium and N the total number of phosphorus atoms per phage particle. Hence, a plot of $\log_{10} s$ *vs.* $(1 - e^{-\lambda t})$, the fraction of all P^{32} atoms decayed by the t^{th} day, should be a straight line with slope proportional to A_0, the relation actually observed experimentally.

We have studied the inactivation by P^{32} decay of five virulent coliphages T1, T2, T3, T5, T7, and of the temperate coliphage λ. All these strains, except the pair T3–T7, are serologically unrelated, differ in their chemical constitution, morphology, genetic structure, and manner of interaction with bacterial host cells. Radioactive stocks of each strain were grown by the procedure indicated above in media ranging in specific radioactivity from 100 to 300 mc./mg. At these specific activities, approximately 0.03 to 0.1 per cent of all phosphorus atoms are present as the P^{32} isotope. The lysates, whose titer usually represented at least a thousandfold increase over the inoculum, were stored at 4°C. in casamino acid–glycerol medium and the number of infective centers assayed from day to day. The results are presented in Fig. 1 in which the logarithm of the fraction of the survivors in the different phage stocks is plotted against $(1 - e^{-\lambda t})$. It is seen that in agreement with equation (2) a straight line survival curve is obtained in every case. The specific death rates αN, having the dimension *lethal atoms per phage* and obtained by dividing the observed slopes of the lines of Fig. 1 by $-1.48 \times 10^{-6} A_0$, are listed in Table I. Control experiments, not shown in Fig. 1, indicated that non-radioactive stocks of all six strains were stable in casamino acid–glycerol medium at 4°C. and that the radioactive lysates had been diluted sufficiently far to avoid inactivation by any external P^{32}. The six phages evidently fall into two classes of sensitivity to P^{32} inactivation. One class, composed of T2 and T5, is characterized by 4.5×10^4 lethal atoms per phage, the value already observed by Hershey *et al.* for T2 and T4. The sensitivity of the other group, comprising T1, T3, T7, and λ, corresponds to 1.5×10^4 lethal atoms per phage. Hence the strains of the second group are only one-third as sensitive to inactivation by decay of P^{32} as those of the first.

Phosphorus Content and Efficiency of Killing.—

The efficiency of killing per disintegration, α, may be calculated from the specific death rate, αN, if the number of phosphorus atoms per infective unit is known. The phosphorus content of each phage strain was, therefore, determined by means of the following procedure, the results of which are listed in Table I.

A stock of each phage was grown in H medium containing P^{32} at a low but accurately determined specific activity. The lysate was clarified and freed of bacterial debris by two low speed centrifugations (10 minutes at 5,000 *g*) and the phage sedi-

mented and washed three times in nutrient broth by high speed centrifugations (60 minutes at 10,000 R.P.M. for T2, T5; 90 minutes at 15,500 R.P.M. for T1, T3, T7). The number of plaque-forming units and the P^{32} content of the purified suspension

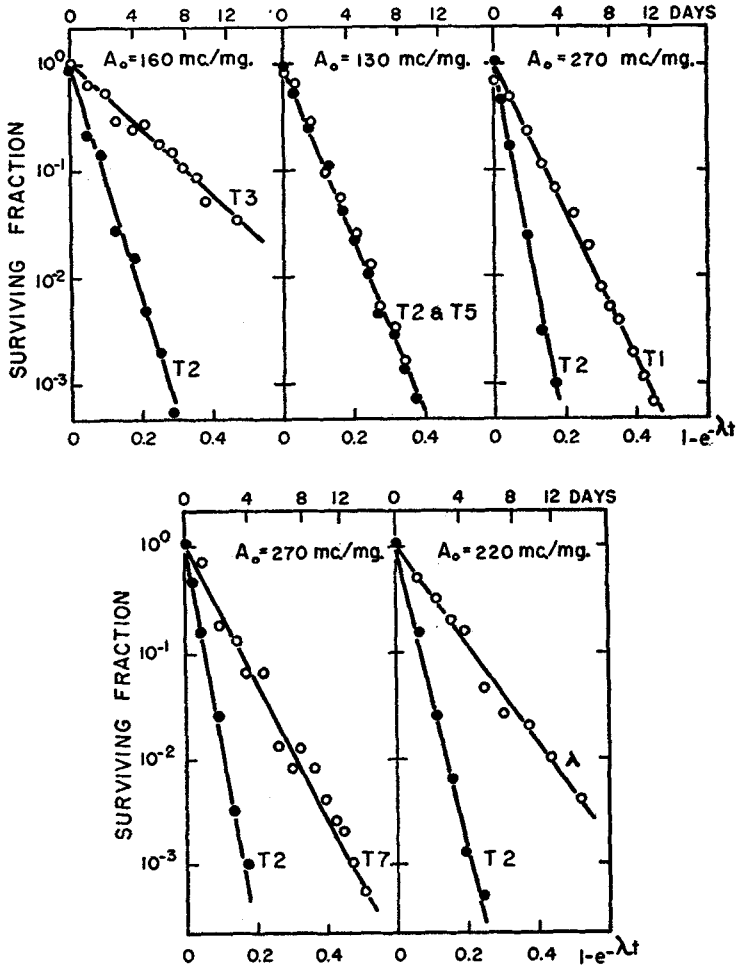

FIG. 1. P^{32} inactivation of T1, T2, T3, T5, T7, and λ at +4°C. A_0 = specific activity of growth medium.

were then assayed and the phosphorus content per infective unit calculated on the basis of the specific activity of the growth medium. In each case, more than 90 per cent of the P^{32} of the purified suspension could be adsorbed specifically to sensitive bacterial cells, indicating that practically all the radioactivity resided in morphologically intact bacteriophage particles. The results of this analysis agree well with the phosphorus content of T2 determined by Hershey, Kamen, Kennedy, and Gest

(1951) and by Hershey and Chase (1952). The agreement is poor, however, with the estimations of the phosphorus contents of T1, T2, T3, T5, and T7 by Labaw (1951) whose values are about twice as great as those found here. No values are listed in Table I for the phosphorus content of λ, since it was not possible to prepare a purified suspension of P^{32}-labelled λ in which the bulk of the radioactivity could be adsorbed specifically to sensitive bacteria. Neither the reason for this behavior of λ nor the nature of the non-adsorbed material has yet been discovered.

The last column of Table I lists the efficiency of killing, α, of P^{32} decay in each of the five strains of T phage. It is seen that in all the strains studied here, α is near the value 0.09 originally observed by Hershey *et al.*; *i.e.*, on the

TABLE I

Evaluation of the Parameters of the Equation

$\log_{10} s = -1.48 \times 10^{-6} A_0 \alpha N (1 - e^{-\lambda t})$

at 4°C.

Phage strain	A_0	Slope of death curve	αN Lethal atoms per phage	P per infective unit	N Atoms of P per phage	α
	mc./mg.			*mg.*		
T2	160*	−10.5	4.5×10^4	2.3×10^{-14}	4.5×10^5	0.10
T5	130‡	−8.1	4.2×10^4	1.8×10^{-14}	3.5×10^5	0.12
T1	270‡	−7.0	1.7×10^4	0.7×10^{-14}	1.4×10^5	0.12
T3	160*	−3.1	1.3×10^4	0.9×10^{-14}	2×10^5	0.07
T7	270‡	−6.4	1.6×10^4	0.9×10^{-14}	2×10^5	0.08
λ	220‡	−4.8	1.5×10^4	?	?	?

* Determined radiochemically.

‡ Determined by comparison with control T2 stock.

average one of about every ten P^{32} disintegrations inactivates any phage particle in which it occurs.

Effect of Temperature on the Efficiency α.—

The rate of inactivation by decay of P^{32} was also measured at two lower temperatures in the frozen state. For this purpose, aliquots of diluted radioactive lysates of all six phage strains were stored either at +4°C., or in the frozen state at −20°C. or −196°C. (the temperature of boiling liquid nitrogen). Samples were then thawed from day to day and assayed for the fraction of surviving infective centers. Frozen controls with corresponding non-radioactive lysates showed that, depending on the strain, from 45 to 90 per cent of the infective centers survive freezing and thawing and that, except in the case of storage of T2 at −20°C., the fraction recovered is independent of the length of time of storage (Sanderson, 1925; Rivers, 1927). It was found that

at these lower temperatures the rate of inactivation by P^{32} decay of all five strains was significantly reduced. Since the rate of radioactive decay is independent of temperature, it follows that a reduction in α by the altered environmental conditions must be responsible for the reduced rate of bacteriophage inactivation. Table II lists the observed values of the slope of the inactivation curves at +4, −20, and −196°C. and the fractional reduction of α compared to its magnitude at +4°C. It is seen that radioactive decay proceeding at −20°C. inactivates the phages with an efficiency of only 70 per cent of decay proceeding at +4°C. Lowering the temperature to −196°C.

TABLE II

The Relative Efficiency of P^{32} Inactivation at Low Temperatures

Phage strain	A_0	Storage at +4°	Storage at −20°		Storage at −196°	
		Slope*	Slope*	$\frac{\alpha(-20°)}{\alpha(+4°)}$	Slope*	$\frac{\alpha(-196°)}{\alpha(+4°)}$
	mc./mg.					
T2	160	−10.5			−6.8	0.65
	130	−8.6			−5.6	0.65
	125	−8.3			−5.7	0.69
T5	130	−8.5	−5.8	0.68	−4.5	0.53
	125	−8.1	−5.6	0.69	−4.6	0.57
T1	270	−7.0	−4.8	0.69	−3.9	0.56
T3	160	−3.1			−1.6	0.52
T7	270	−6.4	−4.6	0.72	−3.6	0.56
λ	220	−4.8	−3.4	0.71	−3.3	0.54

* Refers to the value of $-1.48 \times 10^{-9} A_0 \alpha N$.

reduces the fraction of lethal disintegrations even further. At this temperature the efficiency of killing in T1, T3, T5, T7, and λ is only 55 per cent and in T2 only 65 per cent of its value at +4°C.

Since low temperatures appear to reduce the efficiency α, it seemed possible that radioactive decay occurring at temperatures higher than +4°C. might inactivate bacteriophages with greater efficiency. At elevated temperatures, however, bacteriophages are subject to thermal inactivation, and it is only possible to study the combined effects of heat inactivation and radioactive decay. To examine, therefore, the efficiency α at reasonably high temperatures, a heat-stable mutant, $T5_{st}$, was first selected from our strain of T5 by the procedure of Adams (1953). When stored in glycerol–casamino acid medium at

65°C. a stock of $T5_{st}$ loses 90 per cent of its titer in 5 hours. $T5_{st}$ is inactivated by P^{32} decay at 4°C. with the same specific death rate as the wild type T5. One stock of $T5_{st}$ was grown in H medium containing radioactive phos-

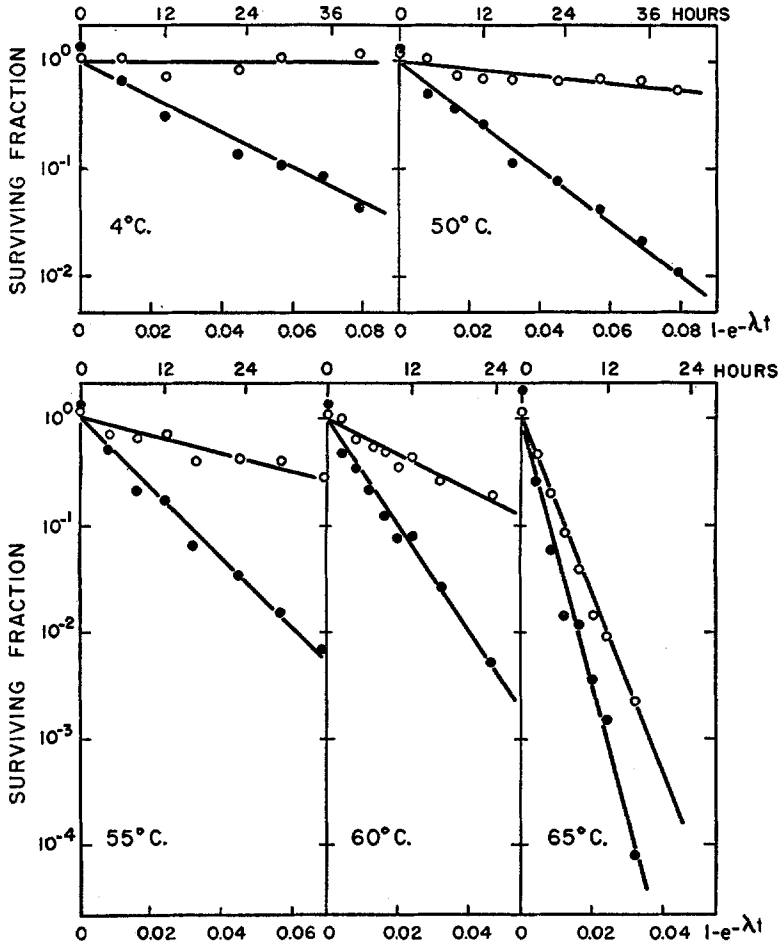

FIG. 2. Inactivation of $T5_{st}$ at different temperatures. Filled circles, radioactive lysate, A_0 = 300 mc./mg. Open circles, non-radioactive control lysate.

phorus at specific activity of 300 mc./mg. (at which level 0.1 per cent of all phosphorus is P^{32}) and one in non-radioactive H medium. After dilution into glycerol–casamino acid medium, aliquots of both lysates were stored at 4, 50, 55, 60, and 65°C. and assays of the number of infective centers made from time to time. The result of this experiment is presented in Fig. 2. It is seen that the rate of inactivation of the radioactive lysate is almost the same at 4,

50, and 55°C., at which temperatures the non-radioactive control lysates exhibited little or no heat inactivation. At 60 and 65°C., however, considerable increases in the rate of inactivation of the radioactive $T5_{st}$ lysate are observed, at which temperatures the non-radioactive control lysate now also exhibits an increasing instability. Since the rate of loss of titer of the radioactive lysate may be presumed to be the sum of the rate of death due to heat and to radioactive decay, the rate of P^{32} inactivation can be estimated at any temperature by subtraction of the slope of the survival curve of the non-radioactive con-

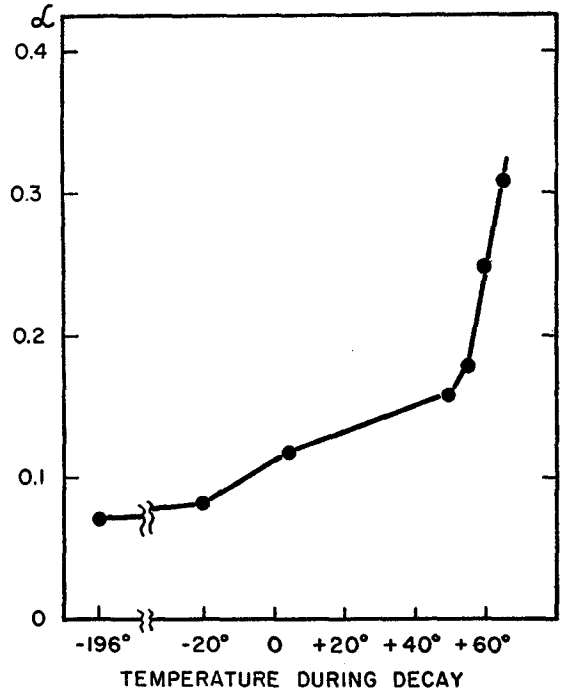

FIG. 3. The efficiency of killing, α, in T5 at different temperatures.

trol from that of the radioactive lysate. (This subtraction of slopes is justified only in experiments of short duration, while $(1 - e^{-\lambda t})$ is still approximated by λt.) The efficiency of killing α at that temperature can then be computed from this difference of rates by means of equation (2). The result of such calculations based on the slopes of Fig. 2 is presented graphically in Fig. 3, in which α has been plotted against the temperature of decay. It is evident that α increases slowly between 4 and 55°C. and begins to rise sharply after that point. At 65°C., α has reached the value 0.31, which means that now almost one in every three P^{32} disintegrations is lethal to $T5_{st}$. Also included in Fig. 3 are the results of the estimations of α in T5 at low temperatures.

Evidently, it is possible to effect at least a fourfold variation in α by varying the temperature of storage from the lowest to the highest practicable range. It is to be noted that the increase in α per degree is greater between -20 and $+4$°C. than between $+4$ and $+50$°C. This, no doubt, implies that α is affected not only by the ambient thermal energy, but also by the change of phase from liquid to solid state.

P^{32} Decay after Infection.—

Hershey and Chase (1952) have shown that when T2 infects a sensitive bacterium, the phosphorus, and hence the DNA, of the bacteriophage particle enters the host cell, whereas the bulk of the phage protein remains outside. It may then be asked whether P^{32} decay can still prevent the reproduction of the parental phage and the ultimate emergence of infective progeny if such decay occurs only after the introduction of the DNA of a radioactive T2 particle into the interior of the bacterial cell.

In order to study the effect of P^{32} decay after infection, it is necessary to arrest intracellular phage development reversibly for days or weeks so that the slow radioactive decay may proceed at an early stage of the brief 20 minute latent period. This can be achieved by quick-freezing the bacterial cells shortly after infection and storing them at -196°C. in liquid nitrogen. As in the case of free phages, non-radioactive controls show that more than half of the infected centers survive freezing and thawing, and that the fraction recovered is independent of the length of storage at -196°C. In those infected bacteria which survive, phage development resumes upon thawing where it had left off at the moment of freezing.

A culture of strain B/r was grown in nutrient broth to a density of 10^8/ml., centrifuged, and resuspended in fresh broth at one-fourth of its original volume. The suspension was then infected with 3×10^7/ml. radioactive T2 particles, containing P^{32} at a specific activity of 88 mc./mg. Phage development was again arrested 2.5 minutes after infection by chilling the culture in ice. The infected bacteria were separated from the small fraction of unadsorbed free phage by centrifugation and resuspended in cold glycerol–casamino acid medium. Aliquots of 0.1 ml. of this final suspension were frozen and stored in liquid nitrogen. From day to day, one of the aliquots was thawed by addition of 1.9 ml. of warm medium and plated at once for the number of surviving infective centers. A control culture infected with non-radioactive T2 under otherwise identical conditions was similarly frozen, stored, and assayed. Aliquots of the initial radioactive stock of free T2 and a non-radioactive control stock were also stored in liquid nitrogen and assayed for their survival from day to day.

The results of this experiment are presented in Fig. 4. It is seen that in the population of bacteria infected for 2.5 minutes with a multiplicity of 0.075 radioactive T2, per cell, the logarithm of the fraction of individuals capable of giving rise to a plaque when plated after thawing decreases linearly with $(1 - e^{-\lambda t})$. The slope of the survival curve is about three-fourth that of the

rate of inactivation of the free radioactive T2 stored at the same temperature. (Neither the control culture infected with non-radioactive T2 nor the

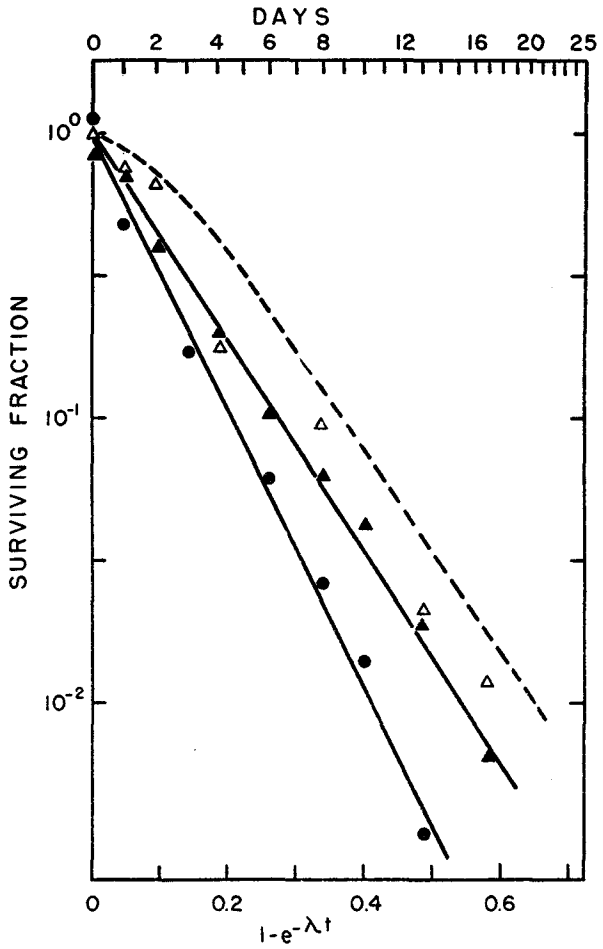

FIG. 4. P^{32} inactivation of T2 (A_0 = 88 mc./mg.) inside infected bacteria at −196°C. Filled triangles, multiplicity of infection: 0.075 (monocomplexes). Open triangles, multiplicity of infection: 2.2 (multicomplexes). Filled circles, free T2. The dashed curve indicates the expected survival of infective centers at a multiplicity of 2.2 in the absence of multiplicity reactivation.

corresponding free phage showed any significant loss of titer.) Hence P^{32} decay occurring in the DNA after it has been separated from the protein "coat" and exchanged its place in the phage head for the protoplasm of the host cell is still capable of destroying the reproductive capacity of the parent phage, although this inactivation now proceeds with a slightly reduced effi-

ciency. Results similar to those presented in Fig. 4 have also been obtained after infection of bacteria with radioactive T3 and λ phages.

State of the Phage after Decay.—

Cross-Reactivation.—The lethal damage sustained by the phage upon decay of one of its phosphorus atoms thus appears to prevent a step of the reproductive cycle which occurs after the invasion of the host. In accordance with this view, we observed that T2 particles inactivated by P^{32} decay are still adsorbed to bacterial cells. In fact, such phages are still able to participate in the reproductive processes occurring inside bacteria infected with a normal, non-inactivated related phage. In experiments already presented elsewhere (Stent, 1953 *b*) it was found that a radioactive stock of the double mutant strain T2hr_1 could still contribute its genetic markers to the progeny of a cross with non-radioactive wild type T2+++ after P^{32} decay had destroyed the ability of the T2hr_1 particles to reproduce themselves *in solo* (*cross-reactivation*). It appeared, furthermore, that the ability of a radioactive T2 particle to donate either one of these two unlinked loci *h* and r_1 is destroyed separately by P^{32} decay, each locus disappearing at about one-third the rate of the plaque-forming ability of the whole particle. In those infected bacteria in which only one of the two radioactive loci has been inactivated, the surviving locus appears among the progeny in nearly normal yield. Stahl (1954) also discovered the existence of cross-reactivation of genetic markers after inactivation of T4 phage by P^{32} decay. Stahl observed, furthermore, that the likelihood that a P^{32} disintegration prevents both of two markers from appearing among the progeny of a cross with an active phage is inversely related to the genetic linkage distance of their loci. Hence it may be inferred that the lethal damage of P^{32} decay affects the reproduction of only part of the hereditary substance of the bacteriophage particle, leaving the rest intact to reproduce itself in mixed infection with an active phage.

Multiplicity Reactivation.—The presence of an active phage particle in the same bacterial cell, however, appears to be necessary for the survival of the undamaged parts of a P^{32}-inactivated T2 phage. Contrary to ultraviolet-inactivated T2 (Luria, 1947), infection of one bacterium by several P^{32}-inactivated particles does not lead to the production of active phage (*multiplicity reactivation*).

In order to test for multiplicity reactivation following P^{32} decay, the stock of radioactive T2 employed in the experiment presented in Fig. 4 was used to infect B/r bacteria at a multiplicity of 2.2 phage particles per cell. As in the low multiplicity experiment of Fig. 4, the mixture of bacteria and radioactive phage was incubated at 37°C. for 2.5 minutes before being frozen, stored at −196°C., and assayed for surviving infective centers from day to day. At a multiplicity of infection of 2.2, the fraction of all infected bacteria to which two or more phages are adsorbed (multicomplexes) is 0.73. Hence if two or more T2 particles were able to cooperate in the

production of active progeny after each individual had already sustained a "lethal" P^{32} disintegration, then the rate of inactivation of 0.73 of the plaque formers in this experiment should have been significantly reduced over the rate of inactivation of singly infected cells. If, on the other hand, the plaque-forming ability of a multiply infected cell is destroyed as soon as each of the infecting particles has been inactivated by P^{32} decay, then the infective centers in this experiment should have disappeared with the "multiple hit" kinetics indicated in Fig. 4 by a dashed curve. The result of this experiment is also shown in Fig. 4. It is seen that inactivation of multicomplexes proceeds at roughly the same rate as inactivation of singly infected bacteria, indicating the absence of any appreciable multiplicity reactivation. Experiments in which P^{32} decay was first allowed to take place in free T2 and in which bacteria were then multiply infected with the inactivated phages likewise failed to reveal any multiplicity reactivation.

Latent Period of Survivors.—Since the efficiency of killing, α, is less than 0.1 at low temperatures, it is apparent that after an amount of decay which

TABLE III

Photoreactivation of T2

Treatment of T2	Assayed in dark	Assayed in light
	Titer	Titer
Before P^{32} decay	1.7×10^8	1.7×10^8
After P^{32} decay	1.7×10^4	1.3×10^4
Before ultraviolet irradiation	2.5×10^9	2.5×10^9
After ultraviolet irradiation	1.4×10^5	1.3×10^7

leaves only a small fraction of the initial phage population still active has taken place under these conditions there have occurred many non-lethal P^{32} disintegrations in the survivors. In the case of T2, these survivors, however, exhibit no evident effects of this non-lethal decay and reproduce with normal latent period and burst size. This is in contrast to the survivors of ultraviolet light irradiation whose multiplication is significantly retarded (Luria, 1944).

Photoreactivation.—T2 bacteriophages inactivated by ultraviolet light can be "photoreactivated" by exposure of bacteria infected with such phages to visible light (Dulbecco, 1949). To examine whether phage inactivated by decay of incorporated P^{32} could be similarly reactivated by light, assays were made of a radioactive T2 stock before and after decay to 0.0001 of the initial titer, incubating the assay plates either in the dark or under a strong fluorescent light. A non-radioactive control stock of T2 was inactivated with ultraviolet light to a survival of 0.000056 and similarly assayed in dark and light. The result of this experiment is presented in Table III, in which it may be seen

that no photoreactivation of the P^{32}-inactivated T2 took place, although the titer of the ultraviolet-inactivated control was raised by nearly a factor of 100 by exposure to visible light.

DISCUSSION

Cause of Death.—

An atom of P^{32} decays into the stable isotope of sulfur, S^{32}, upon ejection of a beta electron–neutrino pair of total kinetic energy 1.7 mev. The beta particle produces ionizations along its path, which are capable of damaging biological materials in a way similar to x-rays. Hershey, Kamen, Kennedy, and Gest, however, showed by means of calculations based on the volume of the T2 particle, the density of ionizations along the beta track and the known efficiency of killing per x-ray ionization, or by reconstruction experiments in which non-radioactive phage particles were irradiated with beta particles emitted by external, non-incorporated P^{32} atoms, that beta particle ionizations could not be the principal cause of the inactivation of radioactive bacteriophage particles. Hershey *et al.* concluded, rather, that a short range consequence of the nuclear reaction, *e.g.* the recoil sustained by the disintegrating nucleus upon ejection of beta electron and neutrino, or the transmutation of phosphorus into sulfur, was responsible for death. The present finding that the sensitivity of radioactive phages to P^{32} decay is reduced only slightly after infection supports this view. For, it appears likely that the state of aggregation of the phage DNA is more compact in the phage head than in the protoplasm of the host cell (Watanabe, Stent, and Schachman, 1954). Hence the chance of irradiation of one part of the phage DNA by distant P^{32} atoms of another would have been seriously reduced once infection was under way.

Efficiency of Killing.—

Hershey *et al.* suggested that the fact that only one P^{32} disintegration in about ten was lethal to T2 or T4 might reflect a division of the phage DNA into 10 per cent "essential" and 90 per cent "non-essential" structures. Under this view, any P^{32} disintegration in the former would be surely lethal and any in the latter generally harmless. The present finding that α is nearly the same in various phage strains of greatly different size, morphology, and biological properties makes this hypothesis less likely. The dependence of α on temperature, furthermore, excludes the possibility that the anatomy of the phage is the sole factor responsible for the efficiency of killing. It seems, rather, that α must at least in part reflect some structural aspect of the DNA molecule, the substance whose function is presumably destroyed by the decay of its radioactive P^{32} atoms.

The lethal effects of P^{32} decay can perhaps be best understood in terms of the macromolecular structure of DNA, recently uncovered by Watson and

Crick (1953), of which a schematic diagram is presented in Fig. 5. This structure reveals DNA as a double helix composed of two intertwined polynucleotide chains of opposite polarity held together laterally by specific hydrogen bonds between purine and pyrimidine bases of opposite strands. The radioactive P^{32} atoms are located in the diester bonds responsible for the continuity of

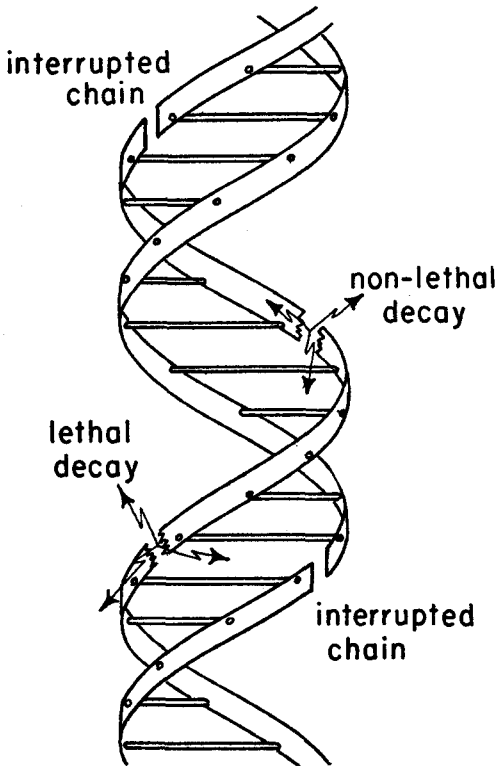

FIG. 5. Schema of the Watson-Crick structure of DNA. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods represent the pairs of bases holding the chains together through a pair of hydrogen bonds. The breaks in the ribbons indicate the spontaneous interruptions of the polynucleotide chains proposed by Dekker and Schachman.

the polynucleotide chains. It appears almost inevitable that every ester linkage is destroyed upon decay of its radioactive phosphorus atom. First of all, the maximum recoil sustained by the phosphorus nucleus is of the order of 80 ev. (the average value being somewhat lower owing to the random orientation of neutrino and beta electron), whereas the energy of the P-O bond holding the atom in place is less than 5 ev. The ester bond is, therefore, probably broken by the Szilard-Chalmers reaction (*cf.* Libby, 1947). Secondly, even if the

recoil does not rupture the phosphate ester linkage, *i.e.* if the phosphorus nucleus remained in place after all, then the two deoxyribose residues are forthwith linked by a *sulfate* diester, which should undergo spontaneous hydrolysis in aqueous medium (Kremann, 1907). Inspection of the structure shown in Fig. 5 indicates, however, that breakage of one ester link would not necessarily lead to the disruption of the DNA molecule, since the multitude of hydrogen bonds still hold the two sister strands together. This has recently been pointed out by Dekker and Schachman (1954), who propose on the basis of physicochemical evidence that the polynucleotide strands of "native" DNA are not actually continuous throughout the length of the macromolecule but are already interrupted in such a fashion that on the average one out of twenty to fifty phosphate links is singly instead of doubly esterified, as indicated in Fig. 5. Thus, if there already exist spontaneous breaks within intact DNA, it is not unreasonable to suppose that the low efficiency of killing per P^{32} disintegration means that the DNA molecule can continue to function even after a few additional interruptions of the polynucleotide chains have been generated by radioactive decay.

An event secondary to the disruption of the phosphate diester must then attend the lethal fraction α of P^{32} disintegrations. The most reasonable hypothesis would appear to be that inactivation is caused by a complete cut of the DNA *double* helix. One way in which this could occur is that enough energy liberated by the decaying P^{32} atom has been transmitted by a sequence of elastic collisions to the other strand to also cause a break there. Another possibility, in view of the proposal by Dekker and Schachman, would be that the lethal decay takes place in an atom situated nearly in apposition to one of the few incomplete ester links on the other strand. In either case, a complete cut results because few or no hydrogen bonds remain between the spots where both sister strands are broken to oppose the dissociation of the macromolecule into two smaller pieces. The effect of heat on the efficiency α is readily explained in terms of this hypothesis. The rapid rise of α above 55°C. must be due to the dissociation of the hydrogen bonds at these temperatures (*cf.* Dekker and Schachman), thus causing less and less resistance to separation of the two strands by the energy of the radioactive disintegration. A greater and greater fraction of the P^{32} decays can, therefore, result in a complete cut of the double helix. The effect of freezing and of low temperatures on reducing α might be explained by the increase of viscosity of the medium in which the two pieces involved in the break have to move; *i.e.*, that when the DNA is embedded in ice there exists a greater chance that the energy of the P^{32} transmutation has already been dissipated before the cut has actually taken place.

Action of Ionizing Radiations.—

It would be possible, though technically rather difficult, to ascertain whether, in agreement with the hypothesis just proposed, decay of incorporated P^{32}

actually depolymerizes highly radioactive DNA molecules with an efficiency similar to α. It is known, however, that x-rays and other ionizing radiations do break down DNA to random fragments of progressively smaller molecular weight at doses comparable to those necessary for the "direct" inactivation of bacteriophages (Taylor, Greenstein, and Hollaender, 1948; Conway, Gilbert, and Butler, 1950). Hence it is not unlikely that the lethal effect of x-ray ionizations inside the phage particle is also one of cutting DNA molecules, similar to that postulated above for P^{32} decay. Two sets of facts would appear to make this comparison useful:

(*a*) The efficiency of killing per x-ray ionization inside the volume of the phage particle is only of the order of 0.05 in the bacteriophage strains studied here (Watson, 1950); *i.e.*, similar in magnitude to α. The energy released by each x-ray ionization is thought to be 32 ev., *i.e.* similar in magnitude to that of the P^{32} recoil, and to be confined to a radius of a few Angstrom units (Lea, 1947). (The average energy available locally may actually be either more or less than 32 ev. because, on one hand, the ionizations tend to occur in clusters but, on the other hand, their energy has been determined only in air and not in a condensed phase.) Since the two polynucleotide chains of the DNA macromolecules are separated by at least 10 A (Watson and Crick, 1953), it would appear possible that many of the ionizations, like many of the P^{32} disintegrations, damage only one of the strands without causing a complete rupture of the double helix.

(*b*) The x-ray sensitivity of T1 depends on temperature very much like α. At temperatures below freezing, the rate of inactivation by x-rays is only 65 per cent of that just above freezing (Bachofer *et al.*, 1953). At higher temperatures, the sensitivity first remains relatively constant and then increases sharply above 50°C., reaching a sixfold greater value at 60°C. (Adams and Pollard, 1952). These observations had already suggested to Adams and Pollard that the weakening of secondary, interchain bonds by heat at the moment of the x-ray ionization might be responsible for increasing the chance of causing lethal damage at higher temperatures. As in the case of P^{32} decay, it is apparent that the greater the extent to which the hydrogen bonds of the DNA macromolecule are dissociated, the more likely will a cut of the double helix result from an energetic rupture of a single polynucleotide strand.

SUMMARY

The inactivation of the phages T1, T2, T3, T5, T7, and λ by decay of incorporated P^{32} has been studied. It was found that these phages fall into two classes of sensitivity to P^{32} decay: at the same specific activity of P^{32} in their deoxyribonucleic acid (DNA), T2 and T5 are inactivated three times as rapidly as T1, T3, T7, and λ. Since the strains of the first class were found to contain

about three times as much total phosphorus per phage particle as those of the second, it appears that the fraction of all P^{32} disintegrations which are lethal is very nearly the same in all the strains. This fraction α depends on the temperature at which decay is allowed to proceed, being 0.05 at −196°C., 0.1 at +4°C., and 0.3 at 65°C.

Decay of P^{32} taking place only after the penetration of the DNA of a radioactive phage particle into the interior of the bacterial cell can still prevent the reproduction of the parental phage, albeit inactivation now proceeds at a slightly reduced rate. T2 phages inactivated by decay of P^{32} can be cross-reactivated; *i.e.*, donate some of their genetic characters to the progeny of a mixed infection with a non-radioactive phage. They do not, however, exhibit any multiplicity reactivation or photoreactivation.

The fact that at low temperatures less than one-tenth of the P^{32} disintegrations are lethal to the phage particle and the dependence of the fraction of lethal disintegrations on temperature can be accounted for by the double stranded structure of the DNA macromolecule.

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