

THE MORTALITY OF BACTERIOPHAGE CONTAINING ASSIMILATED RADIOACTIVE PHOSPHORUS*

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When bacteria and bacteriophage are grown in a medium containing phosphate phosphorus of high specific radioactivity, the viral progeny are markedly unstable. Under suitable conditions one observes a progressive inactivation of the phage that is due solely to the effects of radioactive decay of the assimilated phosphorus. In the range of 30 to 300 atoms of P^{32} per million atoms of total phosphorus (about 10 to 100 curies per gm.), the course of inactivation of the phage can be used for the assay of assimilated P^{32} .

Two kinds of information are obtained in an assay of this kind. The rate of inactivation measures the specific activity of the phage phosphorus, and the form of the survivor curve tells something about the distribution of radioactivity within the phage population. This unique feature of the method may be illustrated in terms of the question which it was designed to answer; namely, whether all or only one of the progeny of a labelled phage particle contains isotopic phosphorus. Evidently the second alternative leads to the expectation that stable individuals will be found among the radioactive phage grown from non-radioactive seed in radioactive medium. We are attempting to apply the method to this and related problems of viral growth. In this paper we report the data on which our method is based, together with some observations having to do with the nature of the lethal reaction.

Materials and Methods

We have used phage T4 propagated on its host *Escherichia coli* strain B, and other closely related phages as noted below.

The tracer sample, either ordinary or "carrier-free" as received from the Oak Ridge National Laboratory, is concentrated by boiling at low pH, neutralized with NaOH,

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centrifuged clear, and incorporated into a medium of the final composition per liter: 0.004 gm. or more phosphorus as orthophosphate, 10 gm. sodium lactate, 5.2 gm NaCl, 1.8 gm. K_2SO_4 , 0.12 gm. $MgSO_4$, 1.0 gm. NH_4Cl , and 0.5 gm. bacto-peptone, at pH 6.8. The peptone contributes 0.0011 gm. of the phosphorus, at least 80 per cent of which is phosphate. The P^{32} content of the medium is assayed in absolute microcuries of beta radiation by means of a Lauritsen electroscope, using a secondary standard of uranium oxide calibrated in turn against a standard solution of P^{32} supplied by the National Bureau of Standards. The total phosphorus of the medium is determined by the method of Fiske and SubbaRow after digestion with sulfuric acid and hydrogen peroxide.

As used below "saline-df" means a diluting fluid containing 0.5 per cent NaCl and 0.001 M $MgSO_4$; "peptone-df" means saline-df containing 0.1 per cent peptone; and "gelatin-df" means saline-df containing 10 mg. per liter of gelatin (Adams, 1948, 1949).

Radioactive phage is prepared by either of two methods, the first being the one-step growth experiment of Ellis and Delbrück (1939) carried out as follows:—An 18 hour bacterial seed culture is prepared by aeration in the above medium containing 5 μ g. P^{31} per ml., and diluted in saline-df to contain about 4×10^7 cells in 0.05 ml. This aliquot is used to seed 1 ml. of the radioactive culture medium, which is aerated for 2 hours at 37°C. There is at least a tenfold increase in cell substance during this time and the final bacterial titer is 10^8 cells per ml. This culture is called the "adsorption tube." The bacteria in the adsorption tube are infected by adding 0.05 ml. of a solution containing 1 mg. per ml. of *L*-tryptophan and sufficient washed phage in saline-df to infect the bacteria with an average of 5 particles per cell. After 5 minutes' further aeration, 90 per cent of the added phage has been adsorbed, and 0.05 ml. of antiphage rabbit serum (diluted 1:5 and dialyzed against saline-df) is added. The concentration of antiserum is chosen to neutralize 95 to 99 per cent of the unadsorbed phage in 5 minutes. Ten minutes after infection the adsorption mixture is diluted 10^3 times ("growth tube 3") or 10^4 times ("growth tube 4") with the radioactive culture medium. Growth of phage and lysis of bacteria take place in the growth tube. At 60 minutes after infection, NaCN to 0.01 M concentration is added to kill any uninfected bacteria, and to prevent any further growth of phage (Doermann, 1948). At 90 minutes after infection, dilutions from the growth tube of 10^5 , 10^6 , and 10^7 -fold with respect to the adsorption tube ("titration tubes" 5, 6, and 7, respectively) are made in peptone-df. The titration tubes are kept below 5°C. for the ensuing period, during which the viable phage titer is measured daily by plaque counts. The yield of phage in these experiments is about 100 per bacterium.

The second, or "lysate" method of preparing radioactive phage consists in infecting the bacteria in the adsorption tube with a very small amount of phage, added together with 30 μ g. *L*-tryptophan per ml. of medium, and allowing the culture to lyse without further dilution or addition. Lysis requires 6 hours or more under these conditions, and the cultures are usually left overnight. The next morning the lysate is centrifuged clear if necessary, and NaCN is added in 0.01 M concentration to the supernate. After 30 minutes longer at 37°C. the lysate is diluted serially in peptone-df, and the resulting "titration tubes," numbered to indicate the logarithm of the dilution, are stored at 5°C. for periodic titration. The principal feature of this method is that it is possible to infect the culture with one phage particle per thousand or more bacteria, so that the contributions from the seed phage of both total phosphorus and viable phage can be

made as small as desired. The yield of phage in this method also is about 100 particles per bacterium.

We have observed the following precautions and controls to ensure that inactivation of phage in the titration tubes is due solely to the effects of assimilated P^{32} .

1. Phage grown by either method in a medium containing P^{32} with a large excess of added P^{31} proves to be stable, showing that the concentrations of antiserum and unassimilated P^{32} in the titration tubes are without effect on the phage. The safe concentration of antiserum has to be determined by trial. The concentration in titration tube 5 is in practice the critical factor limiting the amount to be added to the adsorption tube, and in the conduct of the experiments it has to be remembered that the temperature coefficient of inactivation of phage by antiserum is high (Hershey, 1941). The safe initial concentration of P^{32} (permitting 90 per cent survival) is 26 $\mu\text{c./ml.}$ for 10 days, or 13 $\mu\text{c./ml.}$ for 30 days (see below). These two sources of error are conveniently controlled within each experiment by checking that the titer of phage in titration tube 5 remains 10 times the titer in tube 6 throughout the period of observation.

2. In the one-step procedure, the results obtained using growth tube 3 and growth tube 4 are identical if the concentration of antiserum in tube 3 is not excessive. A delayed effect of antiserum on phage T2Hr1 was encountered, which caused phage from growth tube 3 to be somewhat less stable in the titration tubes than that from growth tube 4. In experiments of long duration with this phage, growth tube 4 had to be used. Growth tube 3 could be used in all experiments with phage T4, which is an advantage because of the lower concentration of P^{32} carried into the titration tubes.

3. In the one-step procedure, a blank run of seed phage plus antiserum without bacteria gives a base line titer of non-radioactive phage below which parallel observations on survival of radioactive phage cannot be carried. This allows a safety factor of 10 if 90 per cent of the seed phage is adsorbed in tubes with bacteria. A delayed effect of antiserum is again seen in the blank, causing progressive inactivation of seed phage in the titration tubes; in this case a convenient circumstance.

Additional controls serve to ensure that the specific radioactivity of the phage will be known from the assay made of the culture medium.

1. Decreasing the bacterial seed five-fold and increasing the period of growth before infection with phage by 1 hour does not affect the rate of death of the phage, showing that equilibration between phosphorus of bacteria and medium is virtually complete under the conditions used.

2. Agreement between the one-step growth method and the lysate method shows that the phosphorus introduced with the seed phage and antiserum is negligible.

3. Identical specific rates of death of phage grown in media with and without

peptone show that the peptone contains negligible amounts of preferentially assimilated phosphorus (see below).

4. Identical rates of death of phage grown in two media of the same specific radioactivity but with different amounts of total phosphorus (for example, 6 and 60 $\mu\text{g./ml.}$) show that the phosphorus contamination from the glassware is negligible. Results described below suggest that a total P content as low as 4 $\mu\text{g./ml.}$ is satisfactory under our conditions, but we have not established the safe lower limit. This becomes important, of course, in experiments requiring high specific radioactivity.

The Relation between Specific Radioactivity of Viral Phosphorus and Survival of Phage

The presentation of data is made easier if we anticipate qualitatively what we have found; the results are compatible with the following assumptions: (1) The inactivation of a radioactive phage particle is the consequence of the disintegration of a single atom (not necessarily the first) of its assimilated P^{32} . (2) At least 999 per thousand of the phage particles are equally sensitive to this type of killing. (3) The P^{32} atoms are distributed at random among the phage particles. It should be observed that we are not concerned with the correctness of these assumptions, which we regard as plausible but essentially unprovable. The useful fact is that populations of the kind we are studying die out in an orderly fashion, which provides a technique for analyzing situations in which assumption (3) does not hold.

From the assumptions stated it follows that surviving phage containing assimilated P^{32} will die at the rate

$$-\frac{dS}{dt} = \alpha N^* \lambda S = 3.4 \times 10^{-6} \alpha N \lambda S A_0 e^{-\lambda t} \quad (1)$$

in which S is the titer of the surviving phage per ml.; α is the efficiency of killing per atomic disintegration; N^* is the number of radioactive and N the total number of phosphorus atoms per phage particle; $\lambda = 0.0485$ per day, the fractional rate of decay of P^{32} ; and A_0 is the initial specific radioactivity of the phage phosphorus in curies per gram. The factor 3.4×10^{-6} comes from the definition of the curie and from λ and converts specific activity to the atomic ratio N^*/N .

For the conditions $S = S_0$ and specific activity = A_0 when $t = 0$, the solution of (1) is

$$\log_{10} S = \log_{10} S_0 - 1.48 \times 10^{-6} \alpha A_0 N (1 - e^{-\lambda t}) \quad (2)$$

It follows that a plot of logarithms of survivors against $(1 - e^{-\lambda t})$ should form a straight line with origin $\log S_0$ and slope proportional to the initial specific radioactivity of the phage phosphorus.

Typical data showing the relation between the specific radioactivity of the medium and survival of the phage T2Hr1 are shown in Fig. 1 and Table I. The phages were grown by the lysate method, excepting sample G which is a one-step growth experiment. The latter is taken from a larger experiment in which adequate samples of the medium could not be spared for determination

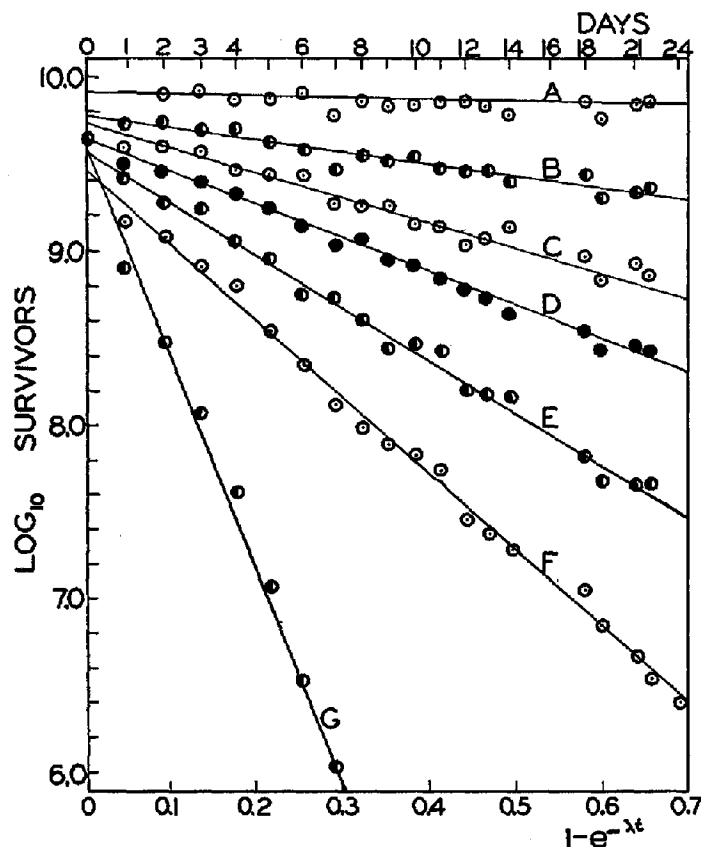


FIG. 1. Survival of phage T2Hr1 containing assimilated P^{32} . The origins of the curves have been adjusted to clarify the graph. See Table I for radioactivity measurements.

of total phosphorus; the specific activity given is approximate. With this exception it may be seen that the proportionality between rate of death of the phage and specific activity of the medium (constancy of αN) is fairly good, and that all the data fit the linear equation (2). The estimates of αN have been computed using equation (2) and the graphically measured slopes of the curves.

TABLE I
Relation between the Specific Radioactivity of the Culture Medium and the Specific Rate of Death of Phage T2Hr1

Curve (Fig. 1)	Initial radioactivity of medium		Specific death rate (αN)
	mc./ml.	mc./mg. P	$\times 10^4$
A	0.27	0.0	—
B	0.14	10.5	4.7
C	0.27	19	5.1
D	0.54	30	4.5
E	1.1	45	4.7
F	2.2	58	5.3
G	0.38	>93	<9.3

TABLE II
Survival of Phage Grown in Radioactive Media under Various Conditions

Curve (Fig. 2)	Method	Medium	[P]	A_0	Log S_0	αN	χ^2
			$\mu\text{g./ml.}$	mc./mg.	per ml.	$\times 10^4$	$\times 10^{-4}$
Media containing depleted peptone							
C	1-step	Lactate-peptone	6.2	25	9.75	4.2	8
H	Lysate	"	6.2	25	9.79	4.2	33
B	1-step	"	15.6	29	9.47	4.3	5
G	Lysate	"	15.6	29	9.90	4.1	8
Media containing whole peptone							
A	1-step	Lactate-peptone	15.6	29	9.32	4.3	7
F	Lysate	"	15.6	29	9.88	4.3	18
Media without peptone							
D	Lysate	Glucose-lactate	5.8	31	10.06	4.3	11
E	Lysate	Lactate	5.8	31	8.93	4.4	17
Media without P^{32}							
—	1-step	Lactate-peptone	5.0	0	9.78	(0.2)	8
—	Lysate	Lactate	1000	0	9.47	(0.4)	13

Figures in parentheses indicate per cent inactivation per day of non-radioactive phage. This datum and the accompanying χ^2 correspond to the equation $\log \frac{S_0}{S} = \frac{kt}{2.3}$. For radioactive phage, χ^2 measures the fit to equation (2). The χ^2 values are directly comparable for the two circumstances.

Similar results have been obtained with 25 samples of radioactive phage, grown in media prepared from five different samples of P^{32} . In these measurements the estimates of αN varied between 41,000 and 58,000, with a mean of

45,000. The data cited include parallel runs with the phages T2Hr1, T2L, T4, and an *r* mutant of T4, which did not differ significantly in radiosensitivity.

The most recent of these measurements are worth describing in further detail because they were designed to test a point of some technical importance, as well as to determine the accuracy of the methods finally worked out.

The point in question arose from the finding of Kozloff and Putnam (1950) that nutrient broth (peptone plus beef extract) contains small amounts of

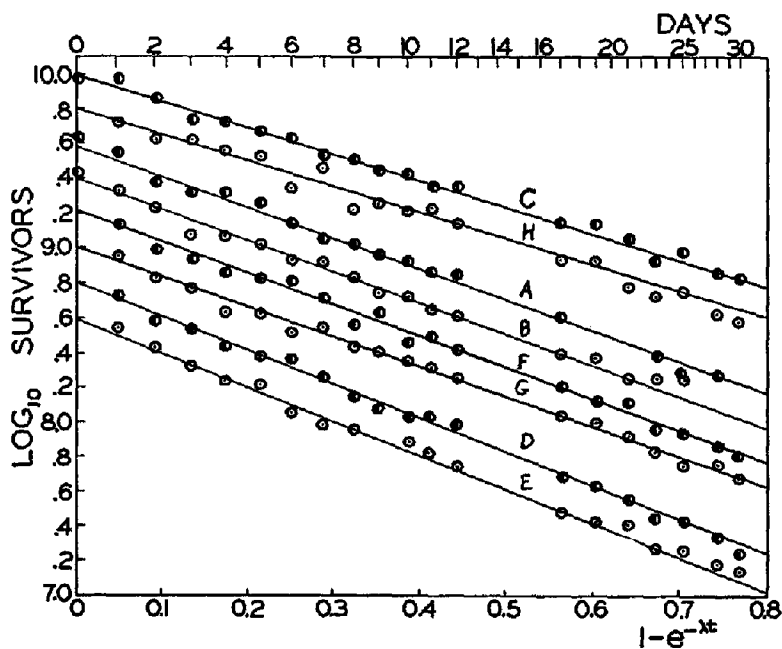


FIG. 2. Survival of radioactive phage T4 grown under various conditions. The lines have been fitted to the data for the first 12 days by the method of least squares. The origins of the curves are arbitrary. The conditions of growth and analysis of data are summarized in Table II.

preferentially assimilated organic phosphorus, causing bacteria and phage grown in broth with added P^{32} to contain phosphorus of lower specific radioactivity than that of the medium as a whole. Similar substances in our medium would introduce serious errors, particularly in the one-step growth procedure in which the culture is diluted with fresh medium during the period in which most of the phage phosphorus is being assimilated.

To test this possibility we have grown phage in media prepared as already described, and with the following variations: peptone omitted, 0.1 per cent glucose substituted for the peptone, and "depleted" peptone substituted for

the whole material. The depleted peptone was prepared following Kozloff and Putnam by allowing bacteria to grow in 1 per cent aqueous peptone to a titer of about 5×10^8 per ml., centrifuging out the cells, and sterilizing the solution. Phage T4 was grown in these media by the one-step and lysate methods at two different concentrations of total phosphorus. The survival of phage was followed as already described, and the data were fitted to equation (2) by the method of least squares. The results are summarized in Table II and Fig. 2. Evidently none of the variations introduced had any effect on the specific rate of death of the phage, and the range of the estimated αN is less than 10 per cent of the mean.

We conclude that radioactive phage dies solely from the effects of the decay of assimilated radioactive phosphorus under the conditions of our experiments, that the specific activity of assimilated phosphorus is the same as that of the medium, and that $\alpha N = 43,000$ (the mean of the data of Table II) is a satisfactory estimate of the specific rate of death of phage T4.

The Phosphorus Content and Volume of the Phage Particle

In order to compute the quantum efficiency of the lethal reaction we wish to know as accurately as possible the phosphorus content of the phages we have used. Published analyses for T4 and related phages (Cohen, 1947; Kozloff and Putnam, 1949) vary upwards from 2.3×10^{-11} $\mu\text{g.}$ per infectious unit. For a staphylococcal phage, Northrop (1938) found 3×10^{-11} $\mu\text{g.}$ We have confirmed the lower estimate, and also have narrowed considerably, we believe, the margin of uncertainty.

In a first experiment phage was purified by centrifugation alone, but with special precautions to control inactivation of the phage during purification. Two lysates were prepared in a medium containing 1 per cent peptone, 0.5 per cent NaCl, 0.1 per cent glucose, and radioactive phosphate. Virtually all the phosphorus in this medium comes from the peptone. The lysates were passed through Sela's porosity 02 porcelain filters. The phage was sedimented and washed three times with peptone-df, followed by two washings with saline alone. Eighty per cent or more of the phage present in the original lysates was recovered in active form in the three times washed concentrate. During the final saline washings, however, 30 to 40 per cent of the activity was lost which could not be found in the supernates. P^{32} was determined on samples of the concentrates after the third and fifth washings, total phosphorus was determined only after the final washing. The results are shown in Table III.

The phosphorus found corresponds to 2.5×10^{-11} $\mu\text{g.}$ per phage particle, and the two phages T2Hr⁺ and T2Hr1 do not differ significantly. For both phages, the five times washed concentrate contains a larger amount of phosphorus per lytic unit, evidently owing to the loss of activity of the phage during washing with saline.

In a second experiment the three phages T2H, T2L, and T4 were grown in a medium similar to the first but containing 0.5 per cent peptone and 15 $\mu\text{g.}/\text{ml.}$ of added phosphorus in the form of phosphate. The phages were purified by sedimentation and three

TABLE III
Phosphorus Content of Phage T2H and Its Mutant r1

	P ³²	Total P	Phage titer	Specific activity	P/phage
	$\mu\text{c.}/\text{ml.}$	$\mu\text{g.}/\text{ml.}$	$\times 10^{11}/\text{ml.}$	$\mu\text{c.}/\mu\text{g.}$	$\times 10^{-11} \mu\text{g.}$
Culture medium	27	28	—	0.96	—
T2Hr ⁺					
3 times washed	8.0	—	2.9	—	2.5
5 " "	7.0	5.5	1.8	1.3	3.5
T2Hr1					
3 times washed	6.0	—	2.5	—	2.2
5 " "	4.0	3.6	1.2	1.1	3.0

The phosphorus per phage is computed from the activity measurement and from the mean specific activity 1.1.

TABLE IV
Phosphorus Content of Several Phages, and Fractionation by Specific Adsorption

Sample	Phage titer	P ³²	P/phage
	$\times 10^{11} \text{ ml.}$	$\mu\text{c.}/\text{ml.}$	$\times 10^{-11} \mu\text{g.}$
T4	2.3	5.4	2.3
T2L	1.1	3.6	3.3
T2H			
Unfractionated	0.68	1.9	2.8
Adsorbed on H (1)	(0.29)	0.79	2.7
Supernate (1)	0.39	1.2	3.1
Adsorbed on H (2)	(0.39)	1.1	2.8
Supernate (2)	0.29	0.90	3.1
Adsorbed on H/2 (3)	0.10	0.29	2.9
Supernate (3)	0.64	1.7	2.7

The phosphorus per phage is computed from the specific activity of the culture medium, 1.0 $\mu\text{c.}/\mu\text{g.}$, containing 31 $\mu\text{g.}/\text{ml.}$ total P.

Phage titers in parentheses are computed by difference.

washings with peptone-df. In addition, samples of the phage T2H were fractionated by adding bacteria (*E. coli* strain H) specifically adsorbing the phage, and separating into adsorbed and unadsorbed fractions by low speed centrifugation. Another sample was fractionated in the same way, except that a mutant strain of *E. coli* (H/2) not adsorb-

ing the phage was used. In this case the phage found in the (unwashed) sediment is largely entrained supernate. All the samples were assayed for P^{32} and for phage. The results are given in Table IV.

The fractionation experiments show that phosphorus is removed from the preparations by adsorption to bacteria nearly in proportion to the removal of titratable phage. The preparations do not, therefore, contain more than a few per cent of the total phosphorus in the form of impurities not specifically adsorbed to bacteria. They also show that if any appreciable amount of phage not titratable by plaque count is present, it is adsorbed to bacteria in the same manner as the titratable phage.

A number of additional preparations of phage were subjected to the action of the enzyme desoxyribonuclease. For this purpose the phage was sedimented and resuspended in veronal buffer of pH 8 containing 0.003 M $MgSO_4$ and 10 $\mu g.$ gelatin per ml. The suspension, containing not more than 10^{11} phage per ml., was divided into two parts, one of which received 20 $\mu g./ml.$ of crystalline desoxyribonuclease (Worthington Biochemical Laboratory) or 200 $\mu g./ml.$ of enzyme prepared according to McCarty (1946). (Either enzyme preparation at a concentration of 10 $\mu g./ml.$ rapidly reduced the viscosity of 0.1 per cent sodium desoxyribonucleate under the conditions specified.) After 2 to 4 hours at 37°C., the phage was clarified if necessary by centrifugation, re-sedimented, and washed and resuspended in gelatin-df. These preparations were analyzed for nucleic acid in terms of the absorption of ultraviolet light at 260 $m\mu$, and for total phosphorus by the colorimetric method. The analytical results for some of the better preparations are shown in Table V.

Evidently the enzyme has no effect either on the infectivity or nucleic acid content of the phage, which again shows a minimal phosphorus content of about 2.5×10^{-11} $\mu g.$ per infective unit. The preparations show significant differences, however. Numerous preparations of T2H grown in synthetic medium showed an extinction coefficient of 1.0×10^{-11} $cm.^2$ per infective unit, whereas the same phage grown in broth usually reads 30 per cent lower. The excessive readings are not due to nucleic acid impurities, because neither elaborate fractional sedimentation nor treatment with desoxyribonuclease has any effect. The variable factor is therefore the efficiency of plating (E) of the phage. Since the best preparations of T2H, T4, and T5 all have about the same nucleic acid content per infective unit, it seems likely that E is near unity in favorable cases. This is an assumption, however, and in what follows we retain E as an undetermined constant in all calculations. Thus the phosphorus content is $2.5 \times 10^{-11} E \mu g.$ per particle, or $N = 5 \times 10^5 E$ atoms.

In other connections we need also an estimate of the volume of the phage particle. According to Taylor (1946), purified phage T2 contains 5 per cent dry weight of phosphorus. From this datum, a phosphorus content of $2.5 \times 10^{-11} E \mu g.$ per particle, an assumed density of 1.15 and water content 38

per cent, the volume is $7.0 \times 10^{-15} E \text{ cm.}^3$, which corresponds to a spherical diameter of $110 E^{1/3} \text{ m}\mu$.

TABLE V
Action of Desoxyribonuclease on Phage

Phage	Preparation No.	Titer	P/phage	E ₂₆₀ /phage
		$\times 10^{11}/\text{ml.}$	$\times 10^{-11} \mu\text{g.}$	$\times 10^{-12} \text{ cm.}^2$
T4-S	1c	7.8	2.7	7.7
"	1e	7.8	2.7	7.4
"	2c	12	2.5	7.2
"	2e	12	2.5	7.0
"	3c	13	2.7	7.2
"	3e	12	3.0	7.4
"	4c	25	3.3	9.0
"	4e	26	3.2	8.5
T4-N	1c	0.69	3.3	9.3
"	1e	0.71	2.9	8.3
"	2c	0.95	2.7	7.7
"	2e	1.2	2.5	7.3
"	3	10	2.5	6.7
T2H-S	1	51	3.5	10
"	2	100	3.4	9.0
T2H-N	1	3.3	2.2	6.6
"	2	1.9	2.6	6.6
T5-S	1	4.4	—	5.0
"	2	3.1	—	6.0
"	3	3.1	—	5.0

T4-S, etc., mean phage grown in a synthetic glucose-ammonia medium.

T4-N, etc., mean phage grown in a medium containing 0.5 per cent peptone, 0.5 per cent NaCl, and 0.1 per cent glucose.

1e is phage incubated with desoxyribonuclease.

1c is the same phage incubated without enzyme, etc.

The titers given refer to independent assays of each sample after preparation for analysis.

E₂₆₀/phage is the extinction coefficient per phage particle at 260 m μ wave length, partially corrected for scattering by subtracting the extinction at 330 m μ .

The Quantum Efficiency of Killing and the Cause of Death

From the estimates already made of $\alpha N = 4.3 \times 10^4$ and $N = 5.0 \times 10^5 E$, where E is the efficiency of our plaque count of T4, the efficiency of killing of phage by decay of assimilated P³² is $0.086/E$ per atom disintegrating. A phage particle dies, therefore, after an average of about 11.6 disintegrations.

When an atom of P³² disintegrates, a beta particle with average kinetic

energy 7.0×10^6 e.v. is emitted, the nucleus absorbs a recoil of the order 30 e.v., and the atom itself is left as sulfur. There is probably an additional local dissipation of energy coming from the rearrangement of electrons in the atom. Any of these effects can be thought of as the cause of death of the phage. The following argument seems to show that the beta radiation is not the cause of death.

A beta particle is supposed to produce its principal biological effects by ionizing atoms along its track. For the beta particles of the P^{32} spectrum the average initial specific ionization is 7.1 ion pairs per mm. of air. Beta particles arising from P^{32} randomly distributed within spherical phage particles of diameter 110μ would traverse an average distance of 4.1×10^{-5} mm. on their way out, which is equivalent to a path in air of 0.04 mm. Thus there are on the average about 0.28 ion pairs produced within the phage by each beta particle emitted. There is, therefore, an average of 11.6 E times this number, or 3.3 E ionizations produced inside the phage particle before it dies. If these ionizations are the cause of death, the efficiency of killing is about 0.3 E per ionization.

The quantum efficiency of killing by beta radiation from P^{32} has been measured directly by mixing phage with radioactive phosphorus in 1 per cent peptone solution, and measuring the rate of death by periodic titration of the mixture. The rate of death under these conditions is expected to be

$$-\frac{dS}{dt} = 6.9 \times 10^{16} maVS = 6.9 \times 10^{16} m_0 aVS e^{-\lambda t} \quad (3)$$

from which

$$\log S = \log S_0 - \frac{6.9 \times 10^{16}}{2.3\lambda} m_0 aV(1 - e^{-\lambda t}) \quad (4)$$

where $m = m_0 e^{-\lambda t}$ is the radioactivity in millicuries per ml., a is the efficiency of killing per ionization within V , V is the volume of the phage particle, and 6.9×10^{16} is the rate of ionization per day per millicurie of radiation. We neglect in this computation any radiation absorbed by or passing through the walls of the tube.

The results of four experiments, in which the titration data are fitted to equation (4) by the method of least squares, are shown in Table VI. The data conform to the equation as shown by the values of χ^2 , and the estimates of aV are in reasonable agreement with each other.

The experiments dated Apr. 3, 1950, in Table VI were done with the same phage and the same sample of P^{32} used in the measurements shown in Table II, and form the appropriate basis for comparison. From the constant $aV = 6.3 \times 10^{-18}$ ml. and the estimated volume of the phage particle given previously, we get an efficiency of killing by beta radiation of 0.009 E per ionization.

This result is not in very good agreement with the data for killing by α -

rays. Latarjet (1948) found, in substantial agreement with Luria and Exner (1941), a mean lethal dose for T2L of 3.4×10^4 r or 5.4×10^{16} ionizations per ml., as compared with our figure of 1.6×10^{17} ionizations per ml. According to our estimate of the volume of the phage particle, the x-ray data correspond to an efficiency of killing of 0.027 *E* per ionization. The discrepancy is unexplained.

Whichever estimate is used, it is clear that the efficiency of killing by beta radiation is too low to account on this basis for the deaths from assimilated P³²; according to our data 34 times too low. We conclude that the inactivation

TABLE VI
Inactivation of Phage by Beta Radiation from P³²

Date	Phage tested	Initial radio-activity	Log <i>S</i> ₀	<i>aV</i>	<i>x</i> [†]
		<i>mc./ml.</i>		$\times 10^{-4}$ <i>ml.</i>	$\times 10^{-4}$
10/12/49	T2Hr1	0.55	8.29	8.9	16
11/14/49	"	0.11	8.30	9.5	15
4/ 3/50	T4	0.68	9.25	6.3	18
4/ 3/50	"	0.34	9.24	6.3	12

The quantity *aV* is a specific death rate equivalent to a "sensitive volume;" $1/aV =$ mean lethal dose in ionizations per ml. All mixtures contain 1 per cent peptone. The phage-P³² mixtures in the experiments of Oct. 12 and Nov. 14 are identical except for intervening decay of P³². Counts in the latter experiment have been corrected for spontaneous inactivation (2.3 per cent per day) of the control phage. There was less than 0.8 per cent per day spontaneous inactivation in the other experiments.

of phage containing assimilated P³² is rarely due to beta radiation, but is usually a direct consequence of the nuclear reaction, which kills with an efficiency of 1/11.6 or a little greater.

DISCUSSION

The finding that the nuclear reaction $P^{32} \rightarrow S^{32}$ kills radioactive phage with an efficiency of about 0.086 per atom transformed presumably means that at least this fraction of the phosphorus atoms of the phage particle is situated in vital structures, and therefore that the vital structures contain nucleic acid.

The relatively low efficiency found might be considered doubtful evidence for the conclusion stated if it were not that the efficiency of killing by ionizations produced in atoms in general is at least as low: about 0.009 for beta rays from P³² and about 0.027 for x-rays, both computed per ionization. These estimates, though precise as stated, are of somewhat doubtful appropriateness because of uncertainty about the quantum of action.

It is tempting to assume from our results that 9 per cent or more of the phosphorus atoms of the phage are strategic in the sense that disintegration of

any one of them kills with high probability, and to relate this to the finding of Putnam and Kozloff (1950) that when a radioactive phage particle infects a bacterium, only 20 to 40 per cent of its phosphorus appears among the viral progeny. Thus one imagines a class of non-essential structures containing about 70 per cent of the phosphorus atoms and subject to metabolic turnover, and a second class containing about 30 per cent of the phosphorus atoms, whose disintegration kills with efficiency about 0.33, and which are conserved during replication of the structures. In this situation our method of assay would measure exclusively the radioactivity of the 30 per cent fraction, and would be competent to deal with the question whether the conserved atoms appear in one or in all of the progeny of a labelled parent.

Our present data exclude the possibility that the conserved atoms appear in only one of the phage progeny. When bacteria growing in radioactive medium are infected each with several non-radioactive phage particles, at least 99.9 per cent of the phage progeny contain essential phosphorus that is radioactive (curve G, Fig. 1). Thus if materially conserved essential structures containing phosphorus exist, it must be supposed that they are dispersed among the progeny during growth, a possibility already suggested by the phenomenon of genetic recombination, in which one sees a functional redistribution of functionally conserved structures during growth (Hershey and Rotman, 1949).

The test of the general hypothesis that materially conserved essential structures exist depends among other things on a test to determine whether the phage progeny as a whole contain all the essential phosphorus that their parents possessed. Our results suggest that this test may now be feasible.

SUMMARY

The bacteriophage T4 containing assimilated radioactive phosphorus is inactivated at a rate proportional to the specific radioactivity of the constituent phosphorus. The beta radiation from the phosphorus makes a negligible contribution to this effect. The inactivation is therefore a direct consequence of the nuclear reaction, which kills the phage with an efficiency of about 1/12. Several phages related to T4 behave similarly.

When radioactive phage is grown from a seed of non-radioactive phage, all of the phage progeny are subject to killing by radioactive decay.

The phage is killed by beta radiation from P^{32} with an efficiency of about 1/100 per ionization within the particle volume.

Bacteriophage T4 and its relatives contain about 500,000 atoms of phosphorus per infective particle. Virtually all this phosphorus is adsorbed to bacteria with the specificity characteristic of the infective particles, and none of it can be removed from the particles by the enzyme desoxyribonuclease. The phosphorus content per particle, together with the published data on

analytical composition, indicates a particle diameter close to 110 $m\mu$ for the varieties of phage studied.

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