THE ORIGIN OF PHOSPHORUS IN ESCHERICHIA COLI BACTERIOPHAGES

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Of the measurements we can make of stages in the growth of bacteriophage from its host cells, one of the most instructive is whether its components are derived from the material of the host or from the medium in which the host is growing. Studies with this end in view have established the fact that for the "wild" even-numbered strains—T2r$^+$, T4r$^+$, T6r$^+$—of bacteriophage against *Escherichia coli*, 20 to 30 per cent of the phosphorus of the bacteriophage was present in the bacteria before their infection with the virus (Cohen, 1948; Kozloff and Putnam, 1950). The other 70 to 80 per cent is derived from the inorganic phosphorus of the medium. Series of experiments have been made to determine whether other strains of bacteriophage have similar ratios of host-to-medium phosphorus. They show that this is indeed not the case but that with T3 and T7, for example, the conditions are reversed and the host bacteria contribute about 80 per cent of the bacteriophage phosphorus.

The relatively large yields obtained of the slowly lysing wild forms of the even-numbered bacteriophages have made it easy to study these problems using radiophosphorus. By using a modified method that requires harvesting relatively small masses of purified bacteriophage, results have now been obtained on the rapidly lysing mutant T2r and on the odd-numbered bacteriophages T1, T3, T5, and T7. Three types of experiment have been made. In those of type I, the inorganic phosphate of the Difco-tryptose broth was labeled with enough P^{32} so that the radioactive content was 10^5 counts per minute per ml of broth.[1] It was then inoculated with enough B strain of *E. coli* to give a bacterial count of about 10^6 cells per ml and incubated at 37 C until the count had reached between 6×10^7 and 1×10^8 bacteria per ml. This culture was multiply infected with bacteriophage in the ratio of 4 to 6 bacteriophage particles per bacterium. The resulting lysate was filtered through a Berkefeld candle about one hour after it had cleared visibly. The bacteriophage in the filtered lysate was purified by a series of three ultracentrifugal sedimentations interspersed with resuspensions and low speed centrifugation in saline. The final dried pellet was assayed for radioactivity. The number of infectious units of bacteriophage in the pellet was computed from the average counts on three independent dilution platings of a measured fraction of the bacteriophage suspension removed just before the final centrifugation. These measurements have yielded the number of counts per minute per infectious bac-

[1] Assuming a counting efficiency of 25 per cent, the absolute radioactive content of the broth would thus be 4×10^5 counts per min per ml or 0.18 microcuries per ml.

teriophage unit obtained by infecting radioactive *E. coli* in the presence of labeled broth. In the experiments of type II, the *E. coli* grown as before in labeled medium was centrifuged and resuspended in warm unlabeled broth immediately before infection with bacteriophage. The subsequent ultracentrifugal purification and the assay for radiophosphorus gave the radioactive count per minute per infectious unit when the bacteriophage was grown on radioactive *E. coli* in unlabeled broth. A comparison between this value and the corresponding one from the type I experiment has yielded the percentage of (labeled) phosphorus in the bacteriophage that had its origin in the bacterium before infection. In experiments of type III, the radiophosphorus was added to the bacterial culture at the same time as the infecting bacteriophage. Comparison between the radioactive count per bacteriophage particle thus obtained and the corresponding one from the type I experiment has furnished the percentage of labeled phosphorus in the

TABLE 1

Phosphorus content of various bacteriophages

BACTERIOPHAGE	P CONTENT PER INFECTIOUS UNIT (g) (TYPE I EXPERIMENT)	P CONTENT PER INFECTIOUS UNIT ORIGINATING IN THE E. COLI BEFORE INFECTION (g) (TYPE II EXPERIMENT)	P CONTENT PER INFECTIOUS UNIT DERIVED FROM MEDIUM AFTER INFECTION (g) (TYPE III EXPERIMENT)	PER CENT OF P FROM E. COLI BEFORE INFECTION $\frac{II}{I}$	PER CENT OF P FROM MEDIUM AFTER INFECTION $\frac{III}{I}$	PER CENT OF P ACCOUNTED FOR $\frac{II + III}{I}$
T1	$2.11 \pm 0.03 \times 10^{-17}$	$1.18 \pm 0.03 \times 10^{-17}$	$0.81 \pm 0.03 \times 10^{-17}$	55.9	38.4	94.3
T2r$^+$	4.36 ± 0.25	1.01 ± 0.04	3.34 ± 0.25	23.2	76.6	99.8
T2r	4.82 ± 0.41	1.54 ± 0.35	3.28 ± 0.41	32.0	68.0	100.0
T3	1.74 ± 0.05	1.35 ± 0.14	0.29 ± 0.04	77.6	16.7	94.3
T5	3.96 ± 0.05	1.21 ± 0.14	2.71 ± 0.01	30.6	68.4	99.0
T7	1.70 ± 0.11	1.32 ± 0.15	0.25 ± 0.03	77.6	14.7	92.3
		Avg. 1.27×10^{-17}g				

All values in the first three columns are averages of three experiments.

bacteriophage derived from inorganic phosphorus in the medium after infection of the host culture.

Table 1 gives average results from the three types of experiment made with the bacteriophages T1, T2r$^+$, T2r, T5, and T7. Data obtained using the slow-lysing T2r$^+$ served as checks against similar results found by other for T2r$^+$ and T6r$^+$. In all cases the radioactive contents per bacteriophage derived from types I, II, and III experiments have been converted into approximate total phosphorus content per bacteriophage by employing the ratio of the labeling inorganic phosphorus to the total inorganic phosphorus content of the broth. This seems justified in view of our findings and of those of Kozloff and Putnam (1950) for T6r$^+$, that over 90 per cent of the phosphorus utilized from the medium under conditions of these experiments was in an inorganic form.

The data from these experiments with T2r$^+$ are compatible with the previous results. This agreement applies to both the total phosphorus and the percentage of virus phosphorus obtained from the radioactive *E. coli* on the one hand and the medium on the other. Thus for comparison with the 4.36×10^{-17} g P per in-

fectious unit for T2r+ in the first column of table 1, Taylor's analysis (Hook, Beard, Taylor, Sharp, and Beard, 1946; Taylor, 1946) leads to about 5×10^{-17} g while Kozloff's (Kozloff and Putnam, 1950) shows a value for the similar T6r+ to be 4.3×10^{-17} g. For comparison with the 1.70×10^{-17} g for T7, recently published analyses (Csáky *et al.*, 1950; Kerby *et al.*, 1949) permit calculation of the three values 1.6, 1.4, and 2.8×10^{-17} g of P per infectious unit.

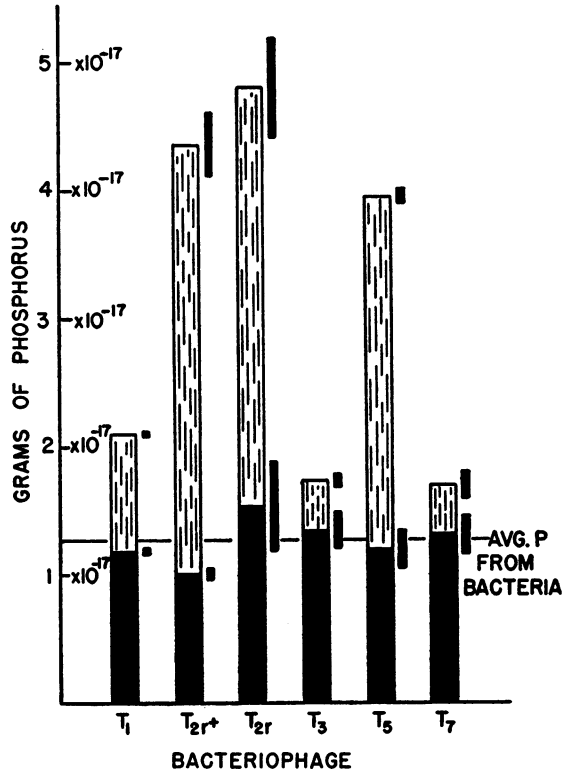

Figure 1. A bar graph of the total phosphorus per lytic unit with the virus phosphorus from the host bacteria superimposed. The narrow bars to the right of the main ones represent the mean deviation in the average values plotted.

Before seeking to interpret these results control runs were made to see if a significant amount of P^{32} was absorbed by the bacteriophage or exchanged with it. In these runs bacteriophage at the maximum concentration prevailing in any experiment was suspended in broth having the standard concentration of P^{32} per ml and then incubated for the maximum time used in any experiment; the bacteriophage sedimented from this suspension and washed with saline was found to contain less than one per cent of the activity per virus particle observed in any of the three types of experiment. Adsorbed or exchanged radioactivity could therefore be neglected.

The data of table 1 show that, as might have been expected, the phosphorus

content of T2r is essentially the same as of T2r$^+$. There seems to be a difference in the source of this phosphorus for the two mutants, but it will require further investigation to establish the significance of this result. The principal source of phosphorus for T3 and T7 is the host bacterium. This is the reverse of the result that T2 and T5 draw most of their phosphorus from the medium. In an intermediary position between these extremes stands the T1 strain.

From the results in the first column of the table, the total phosphorus content per infectious bacteriophage unit is seen to be, except for T5, roughly proportional to the published sizes (Delbrück, 1946) as measured from early electron micrographs. Recent micrographs of T2 and T5 taken in this laboratory under identical conditions indicate that the previous data on T5 are wrong and that its particles are the same size or slightly smaller than those of T2. This brings the results on T5 in line with the others. It is important that this proportionality between the total virus phosphorus and the virus size is supported by chemical analysis of T2, T6, and T7 (Hook, Beard, Taylor, Sharp, and Beard, 1946; Kozloff and Putnam, 1950; Taylor, 1946) which show their nucleic acid contents to be about 40 per cent of their dry weights.

A close study of the data of table 1 indicates that the absolute amounts of virus phosphorus derived from the host before infection and from the medium after infection may have more significance than the ratios of these amounts to the total phosphorus. This is shown by the graphical representation in figure 1 of the data in the first two columns of table 1. Evidently the amounts of virus phosphorus derived from the bacteria before infection (the solid bars in the figure) are the same, within the accuracy of the measurements, for all the types of bacteriophage. This of course suggests that every one of these bacteriophage particles, no matter what its ultimate size, derives from the host a unit of its nucleic acid containing phosphorus already present in the bacterium at the time of infection. If this unit were to exist as a single particle and if it had the composition of desoxypentose nucleic acid, it would weigh about 1.3×10^{-16} g and would therefore have a molecular weight of the order of 80 millions. The present experiments give no indication whether such a particle exists nor do they offer further evidence concerning the significance of this unit of phosphorus.

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SUMMARY

Using a radioactive tracer method the amount of virus phosphorus originating in the host *Escherichia coli* before infection and from the medium after infection was determined for the T1, T2r$^+$, T2r, T3, T5, and T7 bacteriophages. In all of these cases the virus phosphorus having its origin in the bacteria before infection

is constant within the accuracy of the measurements. Since the total phosphorus content per lytic unit is approximately proportional to the size of the bacteriophage, this constant amount of phosphorus already present in the host at the time of infection varies from 20 to 80 per cent of the total phosphorus in the different bacteriophage types.

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