Properties of T4D Bacteriophage Grown in Synthetic Media Containing Zn$^{2+}$, Co$^{2+}$, or Ni$^{2+}$

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Earlier work showed that the tail baseplate of bacteriophage T4D contained 5 to 6 atoms of zinc. T4D bacteriophage now has been grown on Escherichia coli B in a glucose/salts medium containing added $2 \times 10^{-4}$ M Zn$^{2+}$, Co$^{2+}$, or Ni$^{2+}$ ions. Phage particles have also been grown on E. coli in media containing $2 \times 10^{-4}$ M CoCl$\text{$_2$}$. The added zinc and cobalt stimulated the formation of infectious phage particles 2- to 6-fold, while the added nickel depressed the phage yield. The T4D particles formed in the presence of $^{60}$Co were highly purified, osmotically shocked to remove their DNA, and the cobalt content of the ghost particles determined. It was found that there were $4 \pm 1$ atoms of cobalt/ghost particle, which is in reasonable agreement with the previously determined zinc content. The nonradioactive phage particles produced in the presence of these three metals have also been purified and their physical, chemical, and biological properties examined. Properties of these phage particles including heat lability, sensitivity to metal(II) chelating agents such as o-phenanthroline and dimethylglyoxime, and rate of inactivation by Cd(CN)$_2$- treatment varied greatly with the metal ions added to the growth medium. Further, T4D phage grown in the presence of the added Zn$^{2+}$ ions was readily inactivated by partially purified hog kidney folic acid conjugase (a $\gamma$-glutamylpeptidase), phage particles grown in the presence of Co$^{2+}$ or Ni$^{2+}$ ions were completely resistant to this enzyme. The direct analytical data for cobalt, as well as the virion properties, indicate that Co$^{2+}$ and Ni$^{2+}$ ions can be substituted in vivo for Zn$^{2+}$ in the phage baseplate and suggest that the baseplate metalloprotein is physically close to the glutamyl residues of the phage baseplate dihydropteroylhexaglutamate.

Investigations of the properties of the Escherichia coli T-even phage particles continue to be of fundamental interest since they bear on such fundamental questions as (a) the nature and expression of the genetic information needed for the morphogenesis of a complex biological structure and (b) the process by which the viral DNA is efficiently introduced into the host cell. While considerable progress has been made on the morphological features of the virus particles and the changes that take place during infection by Simon and Anderson (1, 2) using refined electron microscopic techniques and by the analysis of the genetic control of morphogenesis in several laboratories (3-8), our understanding of the chemical steps in morphogenesis and the chemical events during injection has developed quite slowly. For example, a critical feature of the distinctly shaped virus particle is the hexagonal baseplate near the terminal end of the phage particle. During interaction with the host cell, this hexagonal structure is known to change its shape from a hexagonal into a star-like structure (1, 2). This morphological rearrangement of a structure whose molecular mass is of the order of $8 \times 10^{10}$ daltons (6-8), results in an opening in the center through which the DNA apparently passes into the host cell (1, 2, 9, 10). The forces and reactants controlling this morphological change, including the nature of the energy needed for the movement of the DNA and its precise introduction into the host cell, are still obscure. Furthermore, the role of the many viral gene products needed to assemble this baseplate structure are only now being appreciated. For example, it is known at least 18 T4 genes are needed to specify proteins to make up the complex baseplate (6-8). Additional features of this complex structure are only now being defined. One unusual feature, which was first reported from this laboratory in 1965 (11-13), was the presence in the baseplate of 6 molecules of an unusual form of folic acid, namely, a dihydropteroylhexaglutamate. Subsequent work revealed that the baseplate also contained two phage-induced enzymes, dihydrofolate reductase (14-17) and thymidylate synthetase (18, 19). The role that these enzymes play either in forming the baseplate or in aiding injection, as well as the presence of an unusual small enzyme cofactor such as folate in the baseplate, has not been linked precisely to the mechanism of the morphogenesis of the baseplate or to its physiological operation. The understanding of the biochemical features of the assembly of this dynamic phage organelle requires not only the determination of which gene products are involved and the sequence of their interaction, but also a knowledge of the bonds holding the structure together and the forces involved in altering its configuration upon interaction with the host cell.

Recently, and somewhat surprisingly, we found that the hexagonally shaped tail baseplates of E. coli T-even bacteriophage particles each contain about 5 to 6 atoms of zinc (20). This conclusion was based on the analytical determination of zinc in T4D, T4B, and T2H phage ghost particles and the
finding that the zinc content of isolated T4D phage baseplates accounted for all the zinc. The specific location of the zinc in the baseplate was confirmed in several ways. It was shown that zinc-chelating agents labilized phage particles to heat (20). The baseplate is the most heat-labile substructure (21, 22) of the phage particle and even minor changes in the structure cause significant changes in the rate of heat inactivation. The 3-fold increase in the rate of heat inactivation caused by the addition of a compound such as o-phenanthroline indicated that zinc probably was a baseplate component. It was also found that the addition of o-phenanthroline and other zinc-chelating agents to mixtures of extracts of cells infected with different T4D mutants markedly influenced specific steps in baseplate morphogenesis.

The role of o-phenanthroline in destabilizing the phage baseplate was shown to be partly due to the removal of baseplate Zn\(^{2+}\) ions. Preparations of T4D particles inactivated with 1 mM o-phenanthroline at 60° could be partially reactivated by a subsequent incubation with Zn\(^{2+}\) ions. Surprisingly, while Zn\(^{2+}\) ions were the most effective metal ions in reconstituting the treated phage particles, Co\(^{2+}\), Ni\(^{2+}\), and to a lesser extent Cd\(^{2+}\), were also able to efficiently reactivate some of the treated particles. T4D particles reactivated with Zn\(^{2+}\), Ni\(^{2+}\), Co\(^{2+}\) or Cd\(^{2+}\) ions had properties reflecting the metal ion used for the reactivation (20).

It is well known that many metalloproteins have their native metal ion removed in vitro and that then other metal ions can be substituted. For example, in the pioneering work in 1958 on carboxypeptidase by Vallee et al. (23), the zinc was removed by treatment with o-phenanthroline and enzymatic activity was regained by reconstitution with Co\(^{2+}\), Ni\(^{2+}\), Fe\(^{2+}\), Mn\(^{2+}\), and even Cr\(^{3+}\), but not with Mg\(^{2+}\), Ca\(^{2+}\), or Cd\(^{2+}\). Since then similar results have been reported for a large number of other enzymes and other metalloproteins. Recent work by Harvey and Barry (24) showed that 2 of 4 zinc atoms of alcohol dehydrogenase can be replaced by cobalt to give a cobalt/zinc hybrid. An additional recent example is the report of Ose and Fridovich (25) who showed that the manganese of superoxide dismutase from E. coli can be removed and that it can be replaced by cobalt, nickel, or zinc.

There have been only a few reports determining whether the in vivo substitution of different metal ions can occur. For mammalian proteins this is difficult, but there is a report by Scruton et al. (26) that the normal Mn\(^{2+}\) ions in chicken liver pyruvate carboxylase can be partially replaced by Mg\(^{2+}\) when the chicklens are maintained on low Mn\(^{2+}\) diets (6). Additionally, Piras and Vallee (27) reported that the zinc of bovine procarboxypeptidase A can be partially substituted in vivo by iron or nickel. For bacterial proteins and even plant proteins, there is the possibility of studying whether these substitutions can occur because the growth medium can be readily altered.

In the experiments reported here, Zn\(^{2+}\), Co\(^{2+}\), including radioactive Co\(^{2+}\), and Ni\(^{2+}\) ions were added to the media in which T4D bacteriophage was grown on E. coli B. The phage particles produced were examined and their baseplates were found to have properties which reflected the specific metal ion added to the medium indicating that the added metal ion was incorporated in vivo into this phage substructure. The direct incorporation of cobalt has been shown to produce phage particles containing 4 ± 1 atoms of cobalt/phage particle. The baseplate metal ions have been found to be closely associated physically with the hexaglutamyl portion of the phage baseplate folic acid (11-13).

**Materials and Methods**

The material and methods were similar to those described in the original report on the presence of zinc in phage baseplates (20). Dimethylglyoxime, reagent grade, was obtained from Merck. It was dissolved in warm 75% ethanol to give a concentration of 5 mM, and when used to treat phage, it was diluted 1:21 in 0.1 M phosphate buffer, pH 6.6, to give a final concentration of 2.3 x 10^-4 M. The hog kidney conjugase preparation was that used earlier (11) which had been partially purified by ammonium sulfate fractionation and by column chromatography on Sephadex G-75. Zn\(^{2+}\) was added as ZnCl\(_2\), Co\(^{2+}\) as Co(NO\(_3\))\(_2\), and Ni\(^{2+}\) as Ni(NH\(_4\))\(_2\)(SO\(_4\))\(_2\)·6H\(_2\)O.

In some experiments, the medium M-9 was supplemented with 1 mM of carrier-free \(^{65}\)CoCl\(_2\) (obtained from New England Nuclear Corp.) and enough carrier CoCl\(_2\) added so that the final concentration was 1.9 x 10^-6 M. Two liters of this medium was inoculated with Escherichia coli and the bacteria grown to 2 x 10^7 ml at 37°C. These cells were infected with T4D so that there was one T4D per three bacteria and the culture shaken for 16 h until lysis was complete. The phage particles were purified in the usual manner, osmotically shocked to release their DNA, and purified further by two cycles of density gradient centrifugation. The linear density gradient was prepared by mixing a light solution of density 1.11 containing 10^-2 M MgSO\(_4\) and 0.01 M phosphate buffer in 99% D\(_2\)O with a heavy solution of density 1.31 in which the light solution was supplemented with 20% CsCl.

**Results**

Growth of Escherichia coli B and T4D in Medium Containing Added Zn\(^{2+}\), Co\(^{2+}\), and Ni\(^{2+}\) Ions—Although E. coli B is known to require Zn\(^{2+}\) ions and other metal ions including iron, copper, and cobalt, these metal ions are not usually added to the standard synthetic bacterial growth medium. It has been reported that the contaminant level of zinc in media prepared with the highest purity reagents is at least 1 to 2 x 10^-8 M which is usually sufficient for normal bacterial growth. E. coli cells will grow in media containing as much as 2.7 x 10^-4 M Zn\(^{2+}\) ions without any noticeable toxicity (28). But Co\(^{2+}\) and Ni\(^{2+}\) ions are quite toxic to cells at such high concentrations (30). The contaminant levels of cobalt, nickel and other metals in typical media have not been reported, but the addition of cobalt or iron ions at the level of 10^-7 M to highly purified media is sufficient to allow maximal growth (28). Nelson and Kennedy (31, 32) have extended the earlier reports that Co\(^{2+}\) ions and Ni\(^{2+}\) ions inhibit E. coli growth (30) and have shown that they are especially toxic in the absence of Mg\(^{2+}\) ions. These authors concluded that both ions competed with Mg\(^{2+}\) transport into the bacterial cell. The glucose/salts media used in these studies, called M-9 (35), contained 1 mM Mg\(^{2+}\) ions so that this toxic effect was minimized.

In initial growth studies of the effect of added Co\(^{2+}\) and Ni\(^{2+}\) ions, it was found that added 5 x 10^-4 M Co\(^{2+}\) or Ni\(^{2+}\) greatly delayed the initiation of bacterial growth; after 48 h, bacterial growth did begin. This prolonged accommodation period of E. coli to a toxic metal ion is similar to that observed for Cd\(^{2+}\) ions (34). However, at 2 x 10^-6 M, Co\(^{2+}\) had no apparent effect on the bacterial growth, and while 2 x 10^-6 M Ni\(^{2+}\) delayed the bacterial growth, after a period of 2 to 3 h of incubation, E. coli growth began at somewhat slower rate and cell cultures reached normal cell concentration levels upon further incubation.

The effect of the addition of Zn\(^{2+}\), Co\(^{2+}\), and Ni\(^{2+}\) ions to M-9 medium on the growth of bacteriophage T4D is shown in Table I. In M-9 with no added divalent metal ions (other than Mg\(^{2+}\)) it can be assumed that the Zn\(^{2+}\) to about 1 to 2 x 10^-7 M (28, 29). The addition of Zn\(^{2+}\) to 1 to 2 x 10^-6 M represented a 10- to 20-fold increase in the concentration of this ion. The
Table I

Effect of addition of metal(II) ions to synthetic media on growth of T4D on Escherichia coli B

The synthetic medium used was the M-9, glucose/salt medium of Adams (33). In all experiments, E. coli B was grown to 5 × 10^9/ml with aeration. In Experiment I, the cells were multiply infected with four T4D/B and then, after 5 min, infected a second time with an additional four T4D/B; in Experiments II and III, the cells were infected with 0.3 T4D/B. In these last two experiments, the infected culture was diluted 10^2-fold after allowing 7 min for adsorption. In Experiment III, the adsorbed phages were determined and the burst size calculated in the standard manner (35). The presence of metal (II) ions did not appear to affect the adsorption rate; approximately 46 to 60% of the added phages formed infectious centers within 7 min in all media examined.

| Metal(II) added | Experiment I | Experiment II | Experiment III |
|-----------------|--------------|---------------|---------------|
| None            | Lysate titer | Lysate titer  | Lysate titer  |
|                 | Lyocase titer| Lyocase titer | Lyocase titer |
|                 | Bursts size (T4/ | cell)         | cell)         |
| Zn^{2+}         | 7.5 × 10^9   | 1.2 × 10^9    | 1.2 × 10^9    |
| 1 × 10^{-4} M   | 2.6 × 10^9   | 3.4 × 10^9    | 2.1 × 10^9    |
| 2 × 10^{-4} M   | 18 × 10^9    | 3.3 × 10^9    | 2.1 × 10^9    |
| Co^{2+}         | 4.3 × 10^9   | 3.3 × 10^9    | 4.5           |
| 1 × 10^{-4} M   | 6.1 × 10^9   | 5.3 × 10^9    | 6.6           |
| 2 × 10^{-4} M   | 36 × 10^9    | 6.6           | 6.6           |
| Ni^{2+}         | 6.8 × 10^9   | 4.7 × 10^9    | 6.5           |
| 1 × 10^{-4} M   | 1 × 10^9     | 2.3 × 10^9    | 2.5           |
| 2 × 10^{-4} M   | 1.3 × 10^9   | 2.3 × 10^9    | 2.5           |

concentration of either Co^{2+} or Ni^{2+} in unsupplemented M-9 is unknown but is probably less than 5 × 10^{-8} M. The addition of Co^{2+} or Ni^{2+} to a final concentration of 1 to 2 × 10^{-6} M would not only greatly increase the concentration of these ions, but would also give a medium in which the molar ratio of Co^{2+} ions (or Ni^{2+}) to the Zn^{2+} ions present would be at least 10 to 20:1.

In all three experiments, the added Zn^{2+} and Co^{2+} ions stimulated phage production, while Ni^{2+} ions depressed the phage yield. Zn^{2+} ions at 1 to 2 × 10^{-4} M roughly doubled the lysate titer and the burst size, while the addition of Co^{2+} ions was even more effective in stimulating phage growth than Zn^{2+} ions, giving yields (and burst sizes) that were 4 to 6 times larger than in the unsupplemented M-9 media. While the added Ni^{2+} ion did not prevent phage growth, at 2 × 10^{-4} M, the phage yields were variable and were only a small fraction of that produced in the absence of added Ni^{2+} ions. Although Cd^{2+} ions gave a smaller amount of in vitro reconstitution of o-phenanthroline-treated T4D phage as compared to zinc, cobalt, or nickel (20), attempts to grow T4D in the presence of 1 to 2 × 10^{-4} M Cd^{2+} were unsuccessful.

The phage in the lysates (of about 20 ml) produced in Experiment I by multiple infection were purified by several cycles of differential centrifugation and finally stored at a concentration of 10^11/ml or higher in saline. These phage stocks were used in the experiments described later. The T4D particles produced in unsupplemented M-9 medium had properties quite similar to those of T4D particles produced in the medium containing the added zinc ions. For example, for the properties examined below, these particles were also quite sensitive to Cd(CN)_4^{2-} and conjugase. The heat sensitivities were also similar, but not identical with T4D/Zn particles. However, in view of the fact that the ion composition of unsupplemented M-9 is unknown and that these phage preparations might be composed of mixed types, detailed data are not presented.

Table II

Heat lability of T4D particles grown in medium containing Zn^{2+}, Co^{2+}, or Ni^{2+} ions

The phage preparations were diluted to a concentration of 10^9/ml in the reaction which contained 0.1 M phosphate buffer, pH 6.6, and 40 μg/ml of gelatin. The error in these determinations is about 10%.

| Phage          | Experiment I | Experiment II | Experiment III | Experiment IV |
|----------------|--------------|---------------|---------------|--------------|
|                |              |                |               |              |
| T4D(Zn)        | 52           | 16            | 6.5           | 49           |
| T4D(Co)        | 51           | 33            | 3.0           | 69           |
| T4D(Ni)        | 31           | 43            | 4.5           | 21           |

It has been known that certain strains of T4 phage particles form plaques on E. coli in different media with quite different efficiencies. These varying efficiencies usually reflect varying adsorption or infection rates. For example, T4B requires the presence of an adsorption cofactor, L-tryptophan, to absorb to its host cell and inject. However, T4B grown in the usual media does not require an adsorption cofactor. Detailed studies of adsorption or injection rates have not yet been carried out for phages grown in various media, but the plating properties of these phages were examined using a variety of plating media. Irrespective of the metal ion in the growth media, the phage particles formed plaques with equal efficiency on broth plates as compared to M-9 plates. The addition of typical adsorption cofactors or inhibitors such as L-tryptophan or indole (35) had little effect on the plating efficiency of these T4D preparations.

Heat Stability of Various T4D Preparations — It has been shown in several laboratories that the phage substructure which is most heat-labile is the phage baseplate (21–23). Any change in this substructure usually alters the rate of phage heat inactivation. It would be expected that the substitution of Co^{2+} or Ni^{2+} for the baseplate Zn^{2+} ions would change the heat sensitivity of the phage particle. In these heat experiments, the inactivation is largely first order and relative heat stabilities can be compared using the time required for 50% inactivation (20). These experiments have all been repeated at least twice with qualitatively similar results. The error in the measurements which is based on change in phage titers is about 10%. It is apparent from Experiment I (Table II) that phages grown in the presence of Ni^{2+} ions (from Experiment I in Table I) and then purified were appreciably more heat-stable than phages grown in media containing Zn^{2+} or Co^{2+} ions.
phages grown in the presence of zinc, while phages grown in the presence of nickel were even more heat resistant than the cobalt-grown phages. These patterns of baseplate sensitivity support the conclusion that these different metal ions are actually incorporated into the tail plate and that zinc can be replaced by cobalt or nickel.

Experiment IV shows the effect of another chelating agent, dimethylglyoxime, under somewhat different conditions on the heat stability of these three phage preparations. Dimethylglyoxime is highly specific for binding nickel ions and is used analytically for this purpose. T4D grown in the presence of Ni\(^{2+}\) ions was highly labilized by this reagent as compared to the effect of this reagent on phage grown in the presence of cobalt or zinc ions.

Martell and Calvin (36) have pointed out that the affinity of almost all chelating agents for these 3 metal ions is nickel > cobalt > zinc, i.e., these agents bind Ni\(^{2+}\) more tightly than Co\(^{2+}\) and in turn bind Co\(^{2+}\) more tightly than Zn\(^{2+}\) ions. However, in the experiments given in Table II, the metal ions are not free in solution, but are part of the protein structure which itself is chelating the metal ion with affinities determined again by the nature of the protein binding groups and by the specific metal. In these experiments, there is competition for the metal ion between the ligand bonds of the protein and ligand bonds of the added agent. In Experiments II and III, it appears that the affinity of the viral proteins for Co\(^{2+}\) and Ni\(^{2+}\) is much higher than their affinity for zinc and, therefore, both o-phenanthroline and 5-nitro-8-hydroxyquinoline inactivated phage containing zinc more readily than phage containing cobalt or nickel. In Experiment IV, the unique and very high affinity of dimethylglyoxime for Ni\(^{2+}\) ions is apparent and this agent rapidly inactivated T4D grown in the presence of Ni\(^{2+}\) ions while the other two phage preparations were less sensitive to this reagent.

Affinity constants for o-phenanthroline and dimethylglyoxime for Zn\(^{2+}\), Co\(^{2+}\), and Ni\(^{2+}\) at 20–25° in various solutions (dioxane or alcohol is used for measurements with dimethylglyoxime) have been gathered by Martell (37). (No affinity constants are available for 5-nitro-8-hydroxyquinoline.) Fig. 1 shows the relationship of these affinity constants and the rate of phage inactivation from Table II. The data in this figure give an indication of the stability constants that this phage substructure has for these 3 different metal ions as compared to the affinity constants for the metal ions in free solution. It is apparent that these metal ions are extremely tightly bound to the phage structure and the affinity is related to the specific metal ion bound in the phage structure. For example, even when heated in the presence of o-phenanthroline, T4D(Ni) is more than twice as stable as T4D(Zn), even though the stability constant of the o-phenanthroline-nickel complex is two logs higher than that of the o-phenanthroline-zinc complex. However, dimethylglyoxime's unique ability to react with protein-bound nickel does compete effectively with the affinity of the nickel ions for the phage proteins.

Inactivation of Various Phage Preparations by Cd(CN)\(_{5}^{2-}\) and Hog Kidney Conjugase—T4D grown in the presence of zinc, cobalt, or nickel was treated with two reagents known to attack the phage baseplate. Cd(CN)\(_{5}^{2-}\) was shown in 1955 (38) to specifically alter phage tail structure. Recently, it has been pointed out that this reagent probably competes for the same protein binding sites as the normal phage baseplate zinc ions (20). Fig. 2 shows the effect of this reagent on purified phage grown in the presence of 2 x 10\(^{-4}\) M zinc, cobalt, or nickel ions. The assay error in this experiment with Cd(CN)\(_{5}^{2-}\) and the experiment described below is about 10%. Zinc-grown T4D was more sensitive to inactivation by this reagent than was cobalt-grown T4D, which in turn was more sensitive than nickel-grown T4D. These results again indicate that these ions can substitute for each other in the phage baseplate; presumably, the added Cd(CN)\(_{5}^{2-}\) competed most effectively with the native zinc and least effectively with the incorporated nickel.

The action of hog kidney folic acid conjugase (a y-glutamyl peptidase) on three different phage preparations is shown in Fig. 3. It has been shown previously that T-even phage...
baseplates contain dihydropteroylhexaglutamate as a structural component (11-13). Phages grown in the presence of cobalt or nickel ions were completely resistant to this enzyme, while T4D grown in the presence of added zinc ions was sensitive. The sensitivity of only T4D(Zn) to hog kidney conjugase has been attributed by Dawes and Goldberg (9) to inactivation of the enzyme during incubation. Not only does this sensitivity of only T4D(Zn) to hog kidney conjugase support the conclusion that cobalt and nickel are incorporated into the different T4D preparations, but it offers the first indication of the structural relationship of the phage baseplate folic acid to the baseplate metal ion. Typically T-even phages are grown in media presumed or known to contain zinc and all have been shown to be sensitive to this enzyme (11-13).

The observation that substitution of cobalt or nickel for zinc alters the baseplate structure so that such phages are resistant to attack by this enzyme not only would be in accord with the fact that cobalt and nickel can efficiently reconstitute phages treated with o-phenanthroline in vitro (20), the evidence here is so specific that it would exclude other interpretations of the properties of phages grown in these different media. These observations include: (a) that the baseplate is structurally different, depending on the metal ion added to the growth medium and that there is a correlation of these structural changes with the chemical properties of the metal ion; (b) that these changes in properties indicate a highly specific location of the metal in the baseplate; and (c) that the amount of cobalt actually incorporated into phage particles is about the same as the amount of zinc found in phages grown in zinc-containing media.

Direct analysis of phage for Ni^{2+} as a replacement for Zn^{2+}...
is difficult even using isotopes or other methods since nickel depresses the phage yield. Spectral evidence for the presence of nickel in uracil has been reported (39), but this optical procedure cannot be used with phage preparations since the observed spectral peaks for nickel in uracil at 316, 425, and 725 nm would require phage preparations for a comparable optical reading to have concentrations of $10^{-6}$/ml, which is about 4 g/ml. Similarly, baseplate preparations would be needed at about 2 to 300 mg/ml for spectral examination, which is also not currently possible. However, the unique effect of dimethylglyoxime in destabilizing nickel-grown phages, but not zinc- or cobalt-grown T4D phages, is strong independent evidence for incorporation of nickel into the phage baseplate.

Some of the biochemical features of this system should be emphasized. Apparently, raising the concentration of cobalt or nickel to $2 \times 10^{-6}$ M in the M-9 medium is sufficient to favor almost the complete exclusion of the normal zinc ions from the virus particle. However, the properties of the phage preparations reported here do not exclude the possibility that a small fraction of the phage particles, possibly 10%, still contains only zinc or even that individual particles may be hybrids and contain various proportions of zinc to cobalt or nickel. The ability to substitute cobalt or nickel for zinc either in vivo or in vitro should aid in determining the physiological role of this metal both in maintaining the phage structure and in facilitating the introduction of the phage DNA into the host cell.

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