Bacteriophage Deoxyribonucleate Infection of Competent \textit{Bacillus subtilis}\footnote{A preliminary report of this work was presented at the 48th Annual Meeting of the Federation of American Societies for Experimental Biology, 12 to 17 April 1964, Chicago, Ill.}

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\textbf{Abstract}

Reilly, Bernard E. (Western Reserve University, Cleveland, Ohio), and John Spizizen. Bacteriophage deoxyribonucleate infection of competent \textit{Bacillus subtilis}. J. Bacteriol. 98:782–790. 1964.—Phenol extracts of the \textit{Bacillus subtilis} bacteriophages \phi 1, \phi 25, and \phi 29 contained infectious deoxyribonucleic acid. The infectivity was destroyed by catalytic amounts of deoxyribonuclease but not by specific phage antiserum, ribonuclease, or trypsin. An infectivity of $>10^4$ infectious centers formed per \mu g of deoxyribonucleic acid (DNA) added was obtained. The stability of the infectious centers permitted an examination of a single cycle of phage replication in cells unable to absorb the mature virus. A typical cycle was observed, although the latent period was increased and the burst size slightly reduced after DNA infection. The development of competence for bacterial transformation was strongly correlated with susceptibility to viral DNA infection. Both appeared and disappeared at the same phase of growth in the cell population. More than 4\% of the viable cells in the competent population were infected by viral DNA. The kinetics of the transition of \phi 29 DNA infection to deoxyribonucleate insensitivity, and the relationship of infectivity to DNA dilution, were similar to the results obtained for bacterial transformation of a single marker. The dose-response curve of \phi 1 and \phi 25 DNA was characteristic of that obtained in multiple transformation of unlinked genetic markers. Because of the low efficiency of infection, about $10^{-4}$ per phage equivalent of DNA added, it was not possible to prove that DNA alone was sufficient to initiate infection.

By demonstrating the functional differentiation of bacteriophage T2 protein and nucleic acid, Hershey and Chase (1952) gave renewed impetus to a search for infectious subviral particles. Each successful exploitation of their discovery necessitated the circumvention of the barrier presented by the normal cell surface. Spizizen (1957) and Fraser et al. (1957) developed the \textit{Escherichia coli} spheroplast as a host, and were able to infect this host with disrupted T2 particles. With \textit{E. coli} C spheroplasts, Guthrie and Sinsheimer (1960) were able to demonstrate the infectivity of purified deoxyribonucleic acid (DNA) of phage \phi X174. A second approach resulted in the infection of intact cells of \textit{E. coli} K-12 by the DNA of the temperate virus \lambda. Kaiser and Hogness (1960) succeeded by simultaneously infecting the \textit{E. coli} host with \lambda DNA and a related "helper" phage. Transformable bacterial cells in the physiological state of competence can incorporate DNA molecules present in solution (Ravin, 1961). Romig (1962) first investigated phage DNA with a transformable host system, using competent \textit{Bacillus subtilis} and a phenol extract of bacteriophage Sp3. Földes and Trautner (1964) extended his observations with \textit{B. subtilis} phage Sp50. Harm and Rupert (1963) investigated competent \textit{Haemophilus influenzae} with the DNA of a temperate virus. Further studies with such host systems have been reported recently (Goodgal, 1964; Pene and Marmur, 1964; Okubo, Stodolsky, and Strauss, 1964; Reilly and Spizizen, 1964).

The data published on the Sp3 (Romig, 1962) and Sp50 (Földes and Trautner, 1964) DNA infections indicate a low efficiency of infection, both on the basis of the amount of DNA added per infectious center formed and with respect to the probable number of competent cells present in the host population. Because of the properties of the infectious centers formed, the extent of the relationship of phage DNA infection to
bacterial transformation is not clear for either infection. In this study the nucleic acids of three _B. subtilis_ bacteriophages have been investigated. The results indicate a significantly greater efficiency of infection and a closer relationship to the _B. subtilis_ transformation system.

**Materials and Methods**

_Bacteria._ _B. subtilis_ H, obtained from Y. Ikeda, was used as a host for lystate production and as indicator strain for the plating of infectious centers. _B. subtilis_ 168 Ind+, the competent recipient strain used by Spizizen (1958), was the parent of the mutants employed in this study. All strains retain the indole marker of the parent. _B. subtilis_ 168M is a highly competent strain of _B. subtilis_ 168, originally obtained from J. Marmur. _B. subtilis_ 168 1°C− is a poorly competent variant isolated by (Young, Spizizen, and Crawford, 1963). Asp 12A is a mutant that is noncompetent, asporo- genic, lacks the ability to produce an antibacterial factor, BH, and is unable to produce a protease.

The symbol ϕ55 refers to a mutation that results in the inability to adsorb ϕ55. The genetic state of this locus or loci has no known effect upon the development of competence for DNA transformation. Strains 168M/ϕ55, 168 1°C−/ϕ55, and Asp 12A/ϕ55 were obtained by plating the parent strain with an excess of ϕ55.

_Transformation assay._ The methods and materials used by Anagnostopoulos and Spizizen (1961) to develop competence and to assay transformation for the indole marker were used in this study.

_Bacterial DNA preparation._ The method of Anagnostopoulos and Spizizen (1961) was used to prepare and purify _B. subtilis_ DNA.

_Chemical determinations._ The colorimetric methods used to quantitate DNA, ribonucleic acid (RNA), and protein were those employed by Spizizen (1958).

_Enzymes and special reagents._ Lysozyme, twice crystallized; ribonuclease, crystallized, ethanol-precipitated; and deoxyribonuclease, once crystallized, were purchased from Worthington Biochemical Corp., Freehold, N.J. Bovine pancreatic type 1 trypsin as a twice crystallized, salt-free ethyl alcohol precipitate was purchased from Sigma Chemical Co., St. Louis, Mo. Liquefied analytical reagent phenol was purchased from Mallinckrodt Chemical Works, St. Louis, Mo.

_Bacteriophage assay._ Bacteriophage were diluted in Difeo antibiotic medium no. 3 (PB). Lysates were stored in PB or a water-salt mixture, termed phage diluent, containing 0.1 m NaCl, 5 × 10⁻² m MgSO₄, and 0.05 m potassium phosphate buffer (pH 7.0). A semisolid medium, composed of Tryptone (Difeo), 5; g; NaCl, 4; g; agar (Difeo), 3; g; and glucose, 3; g; per 500 ml of distilled water, was used as overlay. Sterile glucose (50%; w/v) was added after autoclaving. Plates containing 35 ml of Tryptone Blood Agar Base (Difeo) were used for virus assay as well as for the propagation of bacterial strains.

_Standard bacteriophage techniques._ The methods used in the phage assay (Adams, 1959). Indicator bacteria, _B. subtilis_ H or Asp 12A, were grown in PB at 37°C, with shaking, for use during the log growth phase. Plaques were counted after 18 hr at 37°C. When the log phase contained >2 ml of semisolid medium, a reduction in plaque count occurred. For accurate assay, Tryptose Blood Agar Base plates of less than 2 weeks of age were necessary.

_Lysate production and purification._ All virus preparations were produced and purified by the same general method, which employs standard phage techniques (Adams, 1959). _B. subtilis_ H cells in early logarithmic growth, at 10⁹ cells/ml, were multiply infected (5:1) by phage. Lysis was usually occurred after 4 to 6 hr at 37°C with shaking. No more than 500 ml of PB in a 2.8-liter Fernbach flask were employed, to obtain adequate aeration. Crude lysates usually contained 10⁸ to 4 × 10¹⁰ φ1 or φ25 per ml. The ϕ25 lysates contained 6 × 10¹⁰ to 12 × 10¹² phage per ml. Lysates were clarified by centrifugation at 8,000 × g for 20 min, and were treated with deoxyribonuclease (0.1 μg/ml), ribonuclease (1 μg/ml), and lysozyme (1 μg/ml) at 37°C for 30 min. The virus particles were then sedimented in a Spincu L2 UV ultracentrifuge with a no. 19 rotor at 48,170 × g (maximum) for 3.5 hr. The phage were resuspended in 250 ml of phage diluent and treated with ribonuclease (5 μg/ml) and lysozyme (5 μg/ml) and then with trypsin (5 μg/ml) at 37°C. Debris and clumps were sedimented by centrifugation at 8,000 × g for 20 min, followed by sedimentation of the virus particles at 30,000 × g for 90 min. This cycle of enzyme treatment and differential centrifugation was repeated. The phage were then suspended in phage diluent at 2 × 10¹⁵ to 5 × 10¹⁷/ml. Phage employed for phenol extraction were extracted within 48 hr of storage at 4°C. The conditions reported for the centrifugations were those used for ϕ29. Adjustments were made for the sedimentation of the larger ϕ25 and φ1.

_Phenol extraction of viral DNA._ The procedure of Mandell and Hershey (1960) was used as a guide in the initial steps of the extraction. Liquefied phenol was washed with 0.1 x NaCl in 0.1 m potassium phosphate buffer (pH 7.2) prior to use; 2.5 ml samples of purified virus (2 × 10¹³ phage/ml) in 0.1 x NaCl and 0.1 m potassium phosphate buffer (pH 7.2) were placed in a conical-bottom screw-cap tube. An equal volume of washed phenol was added, and the mixture was chilled in an ice bath. The extract was then centrifuged at 2,000 × g for 10 min at ambient temperature, and the phenol phase was removed with the aid of a capillary pipette. The extract was poured into dialysis bags and dialyzed for 18 hr against 1.5 liters of 0.15 x NaCl and 0.013 x sodium citrate (saline citrate) with stirring at 4°C.
The extract was then transferred to another 1.5 liters of saline citrate, and dialysis was continued for at least 8 hr. A few drops of chloroform were added to retard contamination. The infectivity of the extracts was stable for at least several months in the absence of Mg++ ions.

**Assay for infectious DNA.** Competent cell populations were prepared by the standard method employed in *B. subtilis* transformation. DNA preparations were incubated with competent cells for 45 min at 37°C with shaking. The infected cells were then plated in the standard phage assay, usually with added *B. subtilis* H as indicator bacterium. *B. subtilis* H is not infected by viral DNA. The infection could be terminated by deoxyribonuclease addition at 45 min without any loss of infectivity.

**Antiserum preparation.** Lysates of ϕ1, purified and concentrated by enzyme digestion and two cycles of low- and high-speed centrifugation, were employed as antigen. Adult female white New Zealand rabbits were injected with 0.5 ml of concentrate (10¹⁰/ml) in the ear vein and in each hind foot pad on 3 consecutive days. This series was repeated twice at 7-day intervals. When test bleedings indicated the presence of a high level of neutralizing antibody, the rabbits were bled by cardiac puncture. Blood was stored at 4°C overnight, and the collected serum was heated to 56°C for 30 min and stored at −20°C. When assayed by conventional methods (Adams, 1959), the K value was >1,800. Prior to incubation with infectious DNA, antiserum was diluted 25-fold in 0.1 M NaCl, 5 × 10⁻³ M MgSO₄, and 0.01 M potassium phosphate buffer (pH 7.0), and was heated at 70°C for 45 min to inactivate any deoxyribonuclease present.

**Table 1. Sensitivity of the infectivity of phenol extracts to various agents**

<table>
<thead>
<tr>
<th>Method*</th>
<th>ϕ1 DNA and additions</th>
<th>Infectious centers/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>3.2 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>Antiserum ϕ1</td>
<td>4.1 × 10⁴</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>2.3 × 10⁴ &lt;10</td>
</tr>
<tr>
<td></td>
<td>Deoxyribonuclease (10 μg)</td>
<td>1.4 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>1.6 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>Ribonuclease (10 μg)</td>
<td>2.9 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>Trypsin (10 μg)</td>
<td>2.1 × 10⁴</td>
</tr>
</tbody>
</table>

* (A) The ϕ1 antiserum (diluted 1:10,000) was added to an equal volume of ϕ1 DNA (4 μg) in phase diluent. The control and antiserum tubes were incubated at 37°C for 30 min. The DNA was then tested for infectivity. (B) Enzyme solutions (0.05 ml) were added to ϕ1 DNA (4 μg) in 0.5 ml of phase diluent, and were incubated at 37°C for 20 min. The infectivity remaining was then assayed.

**Results**

A series of *B. subtilis* bacteriophages were isolated from soil by conventional methods (Reilly, unpublished data). These isolates have been differentiated by means of classical bacteriophage techniques and form several distinct groups. Host range, resistant mutants, specificity for neutralizing antibodies, growth-curve parameters, and sensitivity to chemical and physical agents have been employed to delineate the types of phages studied. The virulent bacteriophages ϕ1, ϕ25, and ϕ29 each represent one such group and have served as a source of infectious nucleic acid. Although there are unique features to each viral nucleic acid infection, they are very closely related. Some of the properties of the infectious material are described for only a single nucleic acid source. In those instances, each DNA examined gave a comparable response.

**Some properties of the infectious agent.** Antiserum against ϕ1, sufficient to inactivate >99% of the mature phage present in the control, failed to inactivate the phenol extract (Table 1). However, complete loss of infectivity followed deoxyribonuclease (10 μg) treatment. Trypsin (10 μg) and ribonuclease (10 μg) were without effect upon the infectious nucleic acid. These three enzymes were used in the purification of the bacteriophage without loss of titer. These results suggest that DNA is an active component of the phenol extract and that mature phage particles do not account for the infectivity present. Sodium lauryl sulfate, chloroform, ether, or storage in the presence of citrate inactivated the mature virus but did not reduce the infectivity of the phenol extracts. The complete destruction of activity by deoxyribonuclease contrasts with the absence of a trypsin effect or the absence of inactivation by chloroform or sodium lauryl sulfate. These observations seem to suggest that a complex structure, such as that of the disrupted T2 particle, is not the infectious agent.

Spizizen (1957) observed a loss of infectivity of a preparation of heated T2 when incubated with deoxyribonuclease in the presence of excess citrate. It was suggested that the reduction may have resulted from the presence of contaminating enzymes. To substantiate the conclusion that DNA is critical to this infection and that deoxyribonuclease is the agent of inactivation, the enzyme was tested in catalytic amounts (Table 2). Preincubation with 10⁻⁴ μg of deoxyribonuclease destroyed 99% of the infectivity of the phenol extract. Mature ϕ1 was unaffected.

The ultraviolet absorption of the preparation is characteristic of DNA in solution. A repre-
sentative absorption ratio (260/280 m\(\mu\)) was 1.97. The colorimetric diphenylamine method established the presence of DNA. RNA and protein are present in limiting amounts, representing less than 5\% of the mass of the DNA present. In 7.2 M sodium perchlorate, this material has the melting characteristics of native double-stranded DNA. In CsCl the native and heat-denatured extracts have buoyant densities that are characteristic of viral DNA. (These latter studies were performed in collaboration with W. Szybalski of the University of Wisconsin.)

**Results of a one-step growth curve.** Stable infectious centers result from the interaction of competent cells and viral DNA, and permit the examination of the one-step growth curve. Competent cells of strain 168 M were infected by \(\phi 25\) or \(\phi 25\) DNA. The results presented in Fig. 1 are a composite of several experiments. At 5 min, phage adsorption was terminated by antiserum addition; at 10 min, the culture was diluted 100-fold into prewarmed transformation medium. Uptake of \(\phi 25\) DNA was terminated by the addition of deoxyribonuclease at 20 min, followed by dilution at 25 min. Infectious centers were assayed at 10-min intervals by routine methods. In transformation medium, the \(\phi 25\) lytic cycle had a latent period of about 45 min, with a burst size of 80 to 100 particles. The DNA infection had an extended latent period, 80 to 85 min, and a clear-cut burst was not apparent. These results were also obtained with \(\phi 1\) and \(\phi 29\), although the length of the latent periods differed. This lack of a clear burst may result in part from the fact that <2\% of the cells present are infectable by \(\phi 25\) DNA, whereas the progeny phage can recycle in the remainder of the population. Under these conditions, >90\% of phage \(\phi 25\) can adsorb to 168 M in 5 min. A phage-resistant mutant, unable to adsorb \(\phi 25\) but still able to produce \(\phi 25\) when infected by DNA, was isolated. With strain 168 M/\(\phi 25\) (Fig. 1), a typical latent period was observed with \(\phi 25\) DNA. A single infectious cycle was evident, with a burst size slightly reduced when compared with the \(\phi 25\) infection of 168 M. The characteristic extension of the latent period observed may result in part from the fact that phage adsorption is more rapid than the DNA uptake phase of the nucleic acid infection. It is also possible that the cell populations giving rise to infectious centers are significantly different in their capacity to support virus replication; \(\phi 25\) DNA infection seems to be limited to cells competent for DNA uptake at the time of DNA addition, probably a small frac-

**Table 2. Sensitivity of the infectivity of virus \(\phi 1\) and \(\phi 1\) phenol extract to deoxyribonuclease**

<table>
<thead>
<tr>
<th>Deoxyribonuclease</th>
<th>Mature phage†</th>
<th>(\phi 1) DNA†</th>
<th>DNA inactivation</th>
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</thead>
<tbody>
<tr>
<td>(\phi 25)</td>
<td>1.7 (\times) 10⁴</td>
<td>&lt;10</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>1.5 (\times) 10⁴</td>
<td>&lt;10</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>10⁻²</td>
<td>1.8 (\times) 10⁴</td>
<td>&lt;10</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>10⁻³</td>
<td>2.0 (\times) 10⁴</td>
<td>2.5 (\times) 10²</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>1.5 (\times) 10⁴</td>
<td>3.2 (\times) 10³</td>
<td>99</td>
</tr>
<tr>
<td>0</td>
<td>2.1 (\times) 10⁴</td>
<td>3.2 (\times) 10⁴</td>
<td>0</td>
</tr>
</tbody>
</table>

*Deoxyribonuclease was added to \(\phi 1\) DNA (2 \(\mu\)) or phage \(\phi 1\) in 0.5 ml of phage diluent. After 30 min of incubation at 37 \(C\), competent cells were added to determine infectivity.

† Infectious centers per milliliter.
tion of the population. In contrast, phage φ25 infection is not limited to competent cells.

Role of competence as a limiting factor. Competence, the ability of cells to bind DNA irreversibly and to become transformed, is a genetic property of the 168 M strain of *B. subtilis* (Young and Spizizen, 1961; Young et al., 1963). The state of competence develops in a cell population only during a certain phase of growth (Anagnostopoulos and Spizizen, 1961). In Fig. 2, the transformation to prototrophy indicates the development of competence in the population. At hourly intervals beginning at 30 min, 168 M cells, incubated in the growth medium, were transferred to tubes containing φ29 DNA or host DNA. After 30 min of incubation for DNA uptake, cells were plated for transformation or for infectious centers. The conditions used in this experiment were not optimal for maximal competence, but 0.3% of the viable cells present were transformed to prototrophy at the peak of competence attained. The correlation between the appearance of competence in the population and the appearance of phage DNA-susceptible cells is evident in Fig. 2. With the 168 M strain, the competence for the indole transformation increased by 6 logs during cell growth, and φ29 DNA infectivity followed that rise. Continued incubation resulted in a parallel decrease in both activities.

Strains 168 I-C− and Asp 12A are mutational blocked strains, of independent origin, that cannot develop full competence (Young et al., 1963; Spizizen, Reilly, and Dahl, unpublished data). In these strains the infectivity of φ29 DNA (Fig. 2) is detected at the time and to the extent that the indole transformation occurs.

Strain 168 M, grown in PB or freshly inoculated into competence growth medium, is physiologically noncompetent. These cells are not infected by viral DNA but do support the infection by mature virus.

**Consideration of the dose-response curve.** The dose-response curve is an important characteristic of bacterial transformation systems. Hotchkiss (1957) studied this phenomenon in pneumococcal transformation, and was the first to emphasize its importance.

The dose-response curves represented in Fig. 3 result from a set of serial twofold dilutions for each viral DNA. Competent cells were added to each tube and, after DNA uptake, infectious centers were plated. The broken line represents the slope of the response obtained by transforming the 168 M strain for the indole marker under these conditions. With φ29 DNA, a linear relationship exists between the DNA concentration and the infectivity in the region from about 5 to 0.01 μg. The similarity of the φ29 dose-response curve with that of indole transformation suggests that an infectious center also results from the interaction of a single DNA molecule with the competent cell. Although not clearly illustrated on this curve, a saturation phenomenon exists with viral DNA infection, just as it does with bacterial transformation. Usually, less than 2% of the cell population can be infected by viral DNA, even with DNA present in 20-fold excess of that concentration yielding the maximum on the linear portion of the response curve. In contrast, intact φ29 can infect more than 99% of the cells in an identical population. Excess host or T2 DNA will act as a competitive inhibitor of the virus DNA infection, just as it will inhibit bacterial transformation.

The φ1 DNA and φ25 DNA gave a more complicated response. These curves (Fig. 3) are similar to those obtained for unlinked multiple transformation in the host system.
Kinetics of infection by DNA. A comparison of the kinetics of phage DNA infection and bacterial transformation is presented in Fig. 4. At zero time, limiting amounts of DNA were added to a culture of competent bacteria. At timed intervals, samples were transferred to tubes containing deoxyribonuclease (50 μg), and were subsequently assayed for infection or transformation. The transition of the bacterial marker to the deoxyribonuclease-insensitive phase occurs more rapidly, but the shape of the time course of this transition is similar for each DNA tested. The number of deoxyribonuclease-resistant infectious centers is maximal for each virus by 30 to 40 min, a time well within the latent period for each infection studied. The relative position occupied by each infection on the time axis is a reproducible characteristic of this system.

In Fig. 5, the results of the DNA-uptake experiment are plotted in a manner introduced by Goodgal (1961) in his study of linkage in H. influenzae transformation. With his notation, the number of bacterial transformants $N_t$ at time $t$ is divided by the total number of transformants $N_t$ obtained. When $(1 - N_t/N)$ is plotted against time, a straight line is characteristically obtained for transformation of a single marker. The transition of each viral infection to deoxyribonuclease stability yields a straight line on this plot. A displacement on the time axis occurs. A possible source of this anomaly may be the extremely high molecular weight DNA molecule that must be taken up and protected from deoxyribonuclease to yield an infectious center. The kinetics of infection, as described in Fig. 4 and 5, suggest that infection results from the interaction of a single DNA molecule with a bacterial cell in a manner similar to that postulated for bacterial transformation.
sensitivity of the active agent to deoxyribonuclease and on the correlation that exists between the susceptibility to infection and the presence of bacterial competence for genetic transformation.

DNA preparations used in these experiments gave $5 \times 10^8$ to $10 \times 10^8$ infectious centers per $\mu$g of DNA added to the cells. More recently, specific activities of $>5 \times 10^8$ infectious centers per $\mu$g of DNA have been obtained. This represents a significant increase in specific activity over that obtained with Sp3 or Sp5 (Romig, 1962; Foldes and Trautner, 1964). Kaiser (1962) reported infectivity of $10^6$ per $\mu$g of $\lambda$ DNA added.

The molecular weight of the DNA of $\phi 1$ and $\phi 25$ is near that of coliphage T5, about $10^6$. For $\phi 29$, the DNA molecular weight is approximately $2.5 \times 10^8$, probably less than that of coliphage T7 (unpublished data obtained in collaboration with W. Szymbalski). With these estimates as a guide, the specific activity of the DNA preparations can be expressed in terms of phage equivalents of DNA. In this system, 10,000 to 20,000 phage equivalents of purified DNA yield a single infectious center. Harm and Rupert (1963), with an H. influenzae phage, reported an efficiency of $6 \times 10^{-3}$. This was based upon the number of infectious particles present at the time of DNA extraction, and did not account for loss of infectivity before or during purification; the value may therefore be overestimated. Foldes and Trautner (1964) calculated that $10^7$ infectious centers occur for each Sp50 equivalent of DNA added to the competent B. subtilis. Each infectious viral nucleic acid seems to be characterized by a low absolute efficiency of infection, and this renders impractical a judgment of the sufficiency of DNA as an infecting agent. The limited amount of RNA or protein present could possess functional significance in this type of infection.

Competent B. subtilis cells have an efficient uptake mechanism for native DNA, and under optimal conditions transformation is proportional to this uptake (Young and Spizizen, 1961). Little, if any, RNA or heat-denatured DNA is fixed under comparable conditions. Nevertheless, a rare type of DNA structure or molecular aggregate could form the active infectious agent. In addition, this investigation has not eliminated the possibility that an unknown type of cell in the competent population, perhaps a partial protoplast, could serve as a host for this postulated nucleic acid. However, the existence of a strong correlation between susceptibility to DNA infection and competence for genetic transformation seems to support the hypothesis that native DNA is the infectious agent.

Bacterial strains that have the genetic capacity

**Fig. 5. Kinetics of transformation or DNA infection.** The data in Fig. 4 were used as follows: the number of infectious centers $N_t$ at time $t$ were divided by the total number of infectious centers formed $N_f$; $(1 - N_t/N_f)$ was plotted as a function of time.

**Discussion**

The viral genome, when present as DNA, has become an increasingly important subject for research on the chemical and physical properties of native DNA in solution. Each virus preparation, upon extraction, yields a nucleic acid which is potentially of uniform size and composition. It is now necessary to ask whether a single native DNA molecule is necessary and sufficient to initiate the formation of an infectious center. A key element in an effective biological assay system is the infectious center. The one-step growth curve and the kinetic study of the transition to deoxyribonuclease insensitivity are both made possible by the presence of stable infectious centers. In this study, cells being transformed for the indole marker or being infected by viral DNA seemed to have in common the ability to survive the manipulations of the standard transformation assay (Anagnostopoulos and Spizizen, 1961). Thus, in this viral DNA infection system, a firm basis exists for quantitative studies.

The evidence that DNA is a necessary factor in infectious-center formation is based on the
to become competent and transformed can be infected by viral DNA; other strains cannot. In fact, the physiological state of competence must exist for the detection of DNA infectivity. Frequently, 1 to 2% of the viable cells in a competent population form infectious centers. This proportion is often in excess of the amount transformed for a given bacterial marker. A 4.5% infection by \( \phi 29 \) DNA was the maximum obtained. Mutant cells that develop competence at a low level can be transformed into strains that form a high level of competence (Spizizen, Reilly, and Dahl, unpublished data). A reciprocal transformation also occurs. In each case, viral DNA susceptibility is transferred with altered bacterial competence. Finally, 168 M/\( \phi 25 \) cells that cannot adsorb \( \phi 25 \) can be serially transformed, first by host DNA and then by viral DNA. Although the indole transformation is deoxyribonuclease-insensitive at the time of viral DNA addition, conversion to prototrophy is reduced by over 80%. When deoxyribonuclease is added with the viral DNA as a control, indole transformation is not reduced. In separate control experiments, both transformation and DNA infection occurred at the 1% level. This suggests that a cell that has fixed host DNA can subsequently be infected by phage DNA.

We therefore suggest that a common mechanism exists in the initiation of transformation and DNA infection, at least until DNA is irreversibly bound to the cell. This has been suggested but not firmly established with Sp3 or Sp50 infection because of the low efficiency of infection and the instability characteristic of the infectious centers formed. Harm and Rupert (1963), with \( H. \) influenzae, obtained more positive evidence and suggested a similar hypothesis.

The dose-response curve obtained for \( \phi 29 \) DNA, and the kinetics of transition to deoxyribonuclease insensitivity of the \( \phi 29 \) DNA infection, are also consistent with the previous hypothesis, and again suggest, by analogy to bacterial transformation, that a single native DNA molecule may be infectious.

By these same criteria, \( \phi 1 \) and \( \phi 25 \) DNA give ambiguous results. At high dilution, the \( \phi 1 \) and \( \phi 25 \) dose-response curve suggests either some type of inactivation or a multiple DNA molecule interaction with the cell, or both. Okubo et al. (1964) presented evidence suggesting that more than one DNA molecule can be active in the formation of a single infectious center. However, the results of deoxyribonuclease treatment during uptake (Fig. 4 and 5) indicate a cell-DNA interaction reminiscent of transformation for a single marker. The recently established sensitivity of high molecular weight DNA to shear degradation in dilute solution may account for this apparent discrepancy (Davison, 1959). Since both \( \phi 1 \) and \( \phi 25 \) DNA molecules have molecular weights of the order of \( 10^6 \) (unpublished studies with W. Szybalski), it is likely that the dilution operation results in shear degradation and accounts for most of the loss of activity shown in the dose-response curve.

Why is the bulk of the DNA inactive in this infectious system? With competent bacterial cells, investigation of the uptake of \( \phi 29 \)-labeled DNA suggests that, for every genome equivalent of DNA irreversibly bound, approximately one transformation event occurs for any marker tested (Ravin, 1961). Information on this point is needed for infectious DNA. There are several possible explanations for the low efficiency obtained. Shearing or damage during extraction and manipulation may occur. Aggregation or folding of the DNA in solution may result in loss of activity. A substance other than DNA may also be critical in the formation of an infectious center. Finally, the DNA-uptake mechanism may alter the structure of the DNA molecule so that the normal mode of viral infection operates at low efficiency. These possibilities are under investigation. By changing the extraction technique or the conditions of uptake, it may be possible to increase the efficiency of infection without resorting to extraordinary methods. In any event, when the basis for the low specific activity of the infectious DNA in terms of phage equivalents is understood, the question that initiated this discussion can be answered and the potential utility of the assay system fully realized.

Acknowledgments

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