The Morphology of Staphylococcal Bacteriophage K and DNA Metabolism in Infected Staphylococcus aureus

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SUMMARY

The morphology and dimensions of bacteriophage K particles were determined by electron microscopy. This virus had an icosahedral head (approx. 70 nm diam.) and a long (210 nm) thin (15 nm) contractile tail which terminated in a complex basal appendage. The precise dimensions of the particles were dependent on the negative stain employed. The buoyant densities of the K virus particle and its DNA were 1.479 g/ml and 1.689 g/ml respectively. The DNA had a base composition of 30% G + C, a contour length of 16.1 µm and a calculated mol. wt. of 33 × 10^6.

Staphylococcus aureus (NCTC 9318) as host, the latent period was 25 min, the eclipse period 14 min and the average burst size 60 p.f.u./bacterium. Infection resulted in inhibition of host DNA synthesis and degradation of the bacterial DNA: the products were used for the synthesis of phage DNA. The kinetics of DNA synthesis in infected and uninfected bacteria were examined. There was no initial cessation of DNA synthesis in the infected bacteria.

INTRODUCTION

The bacteriophages of staphylococci have been classified by Rippon (1956) on the basis of their lytic spectrum, serological group and host range. There are 11 serological groups designated A to L. The bacteriophages used in the routine typing of strains of Staphylococcus aureus are in serological groups A, B, F and L and all are derivatives of temperate viruses. A number of virulent staphylococcal viruses are known and they are not restricted to S. aureus strains as host. These polyvalent bacteriophages are in serological groups D, G and H. The morphology of the temperate staphylococcal phages (Bradley, 1963) is different from that of the virulent types. Whereas the former have a polyhedral head (serological groups B, F and L) or cylindrical head (group A) and a long non-contractile tail, the latter have a polyhedral head with a contractile tail (Rosenblum & Tyrone, 1964).

Although several staphylococcal phages are used internationally for typing purposes, these viruses have received little attention with regard to the biochemistry of their replication. As part of a larger programme of investigations of staphylococcal phages, phage K was selected for initial study because the little information available indicated that it was a virulent phage and not a virulent mutant of a wild-type temperate ancestor. It was therefore expected that the results with this virus would not be obscured by unknown residual temperate virus functions.

Bacteriophage K is probably derived from the phage isolated from cultures of the H strain of S. aureus by Gratia (Gratia & de Namur, 1922) and is in serological group D (Rountree, 1949; Rippon, 1956). Its morphology was first studied by Smiles et al. (1948) and Hotchin (1954) using metal shadowing techniques. The adsorption and infection process of

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bacteriophage K and the effect of acridines on its reproduction have been examined by Hotchin (1951) and Hotchin et al. (1952). We wished to know whether bacteriophage K resembled the virulent T-even bacteriophages of *Escherichia coli*, not only in morphology but also in its effect on host metabolism and whether the base composition of its DNA differed from that of the host. We report here on the morphology and physical properties of bacteriophage K and its overall effect on host DNA metabolism. This virus has not been examined previously by negative staining techniques although some other group D viruses have been stained with phosphotungstic acid (Rosenblum & Tyrone, 1964).

**METHODS**

**Phage and bacteria stocks.** Bacteriophage K and the host, *S. aureus* (NCTC 9318), were from the Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT, U.K. Bacteriophage T7 was from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, U.K.

**Media.** For viable counts and plaque assays bottom layers contained 1% (w/v) Davis agar and soft agar overlays contained 0·6% (w/v) Difco-Bacto agar in Oxoid nutrient broth no. 2 (NB). Defined medium 1 (DM1) used to grow the experimental bacteria contained (per 950 ml): 10 g Oxoid casein hydrolysate, 10 mg adenine, 10 mg guanine, 4·5 g KH₂PO₄, 6 mg (NH₄)₂SO₄·FeSO₄·6H₂O, 1 mg nicotinic acid and 1 μg biotin, and was adjusted to pH 7·4 with 1 m-NaOH. After autoclaving, the following were added aseptically: 20 ml 35% (w/v) glucose, 1 ml MgSO₄ (100 mg/ml), 1 ml 1% (w/v) CaCl₂, 10 ml uracil (2 mg/ml), 10 ml thymine (2 mg/ml), 10 ml cytosine (2 mg/ml), 1 ml L-tryptophan (20 mg/ml) and 1 ml thiamine. HCl (5 mg/ml). Defined medium 2 (DM2) used for the ³H labelling of K phage and host DNA was the same as DM1 except that the thymine was replaced with 10 ml deoxyadenosine (5 mg/ml; Denhardt, 1969).

**Infection procedure.** Exponential phase bacteria were used throughout and resuspended in fresh medium (5 x 10⁸ bacteria/ml) before infection with K phage (m.o.i. 5). Five min was allowed for phage adsorption. All incubations were done at 37 °C.

**Preparation and purification of phage stocks.** Unlabelled phage were prepared from cultures grown in NB, infected at a low multiplicity and incubated until lysis was complete. Purification involved precipitation with polyethylene glycol 6000 (Yamamoto et al., 1970), density-gradient centrifugation, and finally dialysis against 0·05 M-tris plus 0·005 M-CaCl₂ pH 7·5 (TC). An identical procedure was used for producing labelled phage except that the growth medium was DM3 containing 2 μCi/ml ³H-methyl thymidine (18 to 25 Ci/mmol, The Radiochemical Centre, Amersham). This produced phage containing about 2 x 10⁻⁴ d/min/p.f.u.

**Determination of latent period, eclipse period and burst size.** The methods were based on those of Ellis & Delbrück (1939). After infection in TC, unadsorbed phage was neutralized with K phage antiserum before setting up the first and second growth tubes in DM1. To determine the eclipse period, each sample for premature lysis was added to 4 vol. 0·05 M-tris, 0·0012 M-EDTA, 0·145 M-NaCl pH 7·4 (TEN) containing 8 units/ml lysostaphin (Schwarz/Mann, Orangeburg, N.Y., U.S.A.; Schindler & Schuhardt, 1965). After 1 h at 4 °C, the preparation was diluted and assayed for p.f.u.

**Extraction of DNA.** The bacteria were collected, resuspended in TEN and lysed with lysostaphin (10 units/ml for 1 h at 4 °C) followed by SDS (0·6%, w/v) and self-digested Pronase (250 μg/ml; Young & Sinsheimer, 1967). The lysates were extracted at 4 °C with phenol and washed three times with ether. Alternatively, after lysostaphin, the preparation received 1% (v/v) Triton X-100 and was incubated overnight at 4 °C: these lysates were not extracted with phenol.

**Base composition of K DNA.** The DNA was hydrolysed in 0·3 M-HClO₄ in a sealed tube at 100 °C for 60 min, the mixture neutralized with KOH and the KClO₄ removed. Ascending
thin-layer chromatography (TLC) was carried out on plates coated with cellulose with a solvent of methanol–HCl (specific gravity 1.18)–water (70:20:10, by vol.; Randerath & Randerath, 1967).

Buoyant density determinations. Labelled phage K (3 × 10⁷ p.f.u./ml) was centrifuged to equilibrium in a CsCl gradient (initial refractive index, nD° = 1.3791) for 21 h at 100000 g in a Beckman SW41 rotor at 25 °C. Labelled DNA (3 ng/ml) was treated similarly except that initially nD° = 1.3984 and it was centrifuged at 75000 g for 42 h in an SW39 rotor at 25 °C. After fractionation, the refractive indices of selected fractions were measured at 25 °C and converted to densities by using standard tables.

Electron microscopy. Phage particles were picked up on carbon-coated formvar grids and stained with unbuffered 1% (w/v) uranyl acetate (UA) or 2% (w/v) phosphotungstic acid (PTA) pH 6.8. For DNA molecules, the phage (K alone or mixed with T7 at about 1 × 10¹⁰ p.f.u./ml) was treated with neutralized NaClO₄ to release the DNA (Freifelder, 1967) and the samples loaded on to grids using the micro-diffusion procedure of Lang & Mitani (1970), stained with UA, dried and rotary shadowed with platinum/palladium (Davis et al., 1971). Preparations were examined in an AEI EM6B or an AEI Corinth electron microscope. The magnification was frequently calibrated using a carbon replica of a cross-lined optical grating (2160 lines/mm: Agar Aids, Stansted, Essex, U.K.).

Sucrose gradients. Linear sucrose gradients (5 to 20%) in 0.1 M-tris, 0.001 M-EDTA, 0.1 M-NaCl pH 8, were formed over a cushion of 50% sucrose (1 ml). The sucrose concentrations of selected fractions was measured in a calibrated Abbé refractometer. Fraction no. 1 always corresponds to the bottom of the gradient.

Measurements of radioactivity. Measurements were made in a Nuclear Chicago Isocap liquid scintillation counter. Where appropriate, results were corrected for non-specific adsorption of unincorporated ³H-thymidine to Millipore filters and retained bacterial material.

RESULTS

Bacteriophage K morphology

When stained positively with UA, the phage head appeared as a regular hexagon (Fig. 1). This geometric shape was often lost in negatively stained phages. The diam. of the head was 66 nm in preparations stained with UA, and 77 nm when stained with PTA. The tail of the bacteriophage was long (225 nm in UA, 191 nm in PTA) and thin (14 nm in UA, 16 nm in...
PTA). It appeared to be joined to the head by a constrictor neck region, and the far end terminated in a basal tuft (Fig. 2). The basal appendage was poorly defined in UA and seemed to consist of fibre-like structures forming a nearly spherical tuft about 42 nm in diam. Some of the fine structure of the outer layer of the tail sheath was resolved in a number of preparations positively stained with UA (Fig. 2), and it was seen to consist of 50 repeated annuli, apparently lying perpendicular to the long axis of the tail.
In a small number of the virions stained with UA, and the majority of those stained with PTA, the tails were contracted, revealing that the tail of K phage was constructed from an outer cylindrical sheath surrounding a central core (Fig. 3). Contraction of the sheath reduced its length by about half (to 102 nm in UA and 97 nm in PTA) and increased its diam. (to 25 nm in UA and 21 nm in PTA). The tail core protruded beyond the end of the contracted sheath to a length of about 93 nm (UA and PTA), and had a diam. of 8 nm (UA) or 7 nm (PTA). Although the annuli were readily resolved in contracted tail sheaths, they were much harder to identify in contracted tails. When they could be seen in contracted tails, it appeared that their number had been halved to about 25. In PTA-stained preparations the average diam. of the basal tuft was 57 nm and some details became visible: five or six knob-like appendages could be seen, possibly arranged around the perimeter of a base plate or joined by short stalks to the end of the sheath (Fig. 3). In contracted tails this basal tuft remained attached to the end of the contracted sheath and was not associated with the distal end of the core. The dimensions of the phage particles measured in this work are summarized in Table 1.

The three-dimensional shape of the phage head could not be unequivocally inferred from stained preparations because of the compression and flattening of the specimen which occurs as the air–water interface passes through it during drying (Anderson, 1952; Williams, 1953). To avoid this drying effect a preparation of purified K phage particles was applied to an electron microscope grid, immediately freeze-dried and subsequently shadowed with platinum/palladium from one direction only. The shapes of the shadows cast by the phage heads (Fig. 4) were typical of those of a three-dimensional solid having icosahedral symmetry (Williams & Smith, 1958; Nermut & Frank, 1971).

### Buoyant densities of phage K and its DNA

The buoyant density of phage K was found to be (from five determinations) 1.479 (s.d. ± 0.0002) g/ml, and that of K phage DNA was (from 11 determinations) 1.689 (s.d. ± 0.006) g/ml. The phage particle buoyant density falls within the range of 1.474 to 1.483 g/ml previously determined for four other phages in serological group D (Rosenblum & Tyrone, 1964).

### Base composition of K phage DNA

The bases present in a hydrolysate of the K phage DNA were identified by TLC as adenine, guanine, thymine and cytosine by comparing their mobilities with those of standard bases chromatographed on the same plate. No evidence was found for the presence of any
'unusual' bases such as hydroxymethylcytosine or hydroxymethyluracil.

The base composition of the DNA was calculated from its buoyant density in caesium chloride using the relationship derived by Schildkraut et al. (1962) and was found to be 30% G + C. This can be compared with the range of 31 to 36% G + C reported for six typing phages from serological groups A, B, F, and L by Pariza & Iandolo (1974).

Contour length and mol. wt. of K phage DNA

The DNA was prepared for electron microscopy by the method of Lang & Mitani (1970), and measuring errors caused by physical pressures on the DNA molecules were minimized as far as possible by the inclusion of a calibrating DNA. Bacteriophage T7 DNA was chosen to calibrate the system because its contour length (12.15 μm) and molar linear density (2.07 × 10^{10} daltons/cm) have been accurately determined by Lang (1970) who used methods comparable with those employed in this study.

The K phage and T7 DNAs were prepared for electron microscopy either individually or in mixed samples, and over a period of several months no appreciable variations in their respective contour lengths, determined from a number of preparations, were detected. From 18 determinations, the contour length of T7 DNA was found to be 12.0 (s.d. ± 0.5) μm, the mean value being 1.2% below the published length. This apparent underestimation of the DNA contour length was taken into account when calculating the length of the K phage DNA molecule which, from 43 determinations, was found to be 16.1 (s.d. ± 2.6) μm. The mol. wt. of phage K DNA, calculated from its contour length using the molar linear density value of T7 DNA (Lang, 1970), was 33 (±5) × 10^{6}.
The self renaturation procedure of Lee et al. (1970) was used in attempts to determine whether or not the DNA of phage K was circularly permuted or possessed single-stranded terminal redundancies. Examination of DNA treated in this manner did not reveal any circular structures or molecules greater than unit length, which suggests that K phage DNA does not possess circular permutation or cohesive ends, but does not exclude the possibility that the DNA is terminally redundant.

**Cations for adsorption of K phage**

Although there is some adsorption of K phage in 0.01 M-tris buffer pH 7.5 alone, optimum adsorption requires added divalent cations such as Ca$^{2+}$, Sr$^{2+}$ or Mg$^{2+}$. Mn$^{2+}$ ions are not effective, unlike phage λ where Mg$^{2+}$ can be replaced by Mn$^{2+}$ (Fry, 1959). Optimum adsorption of K phage occurred in 0.01 M-tris buffer pH 7.5 containing 0.01 M- or 0.005 M-CaCl$_2$, e.g. at an m.o.i. of 0.1, 40% of the phage were adsorbed in 5 min compared with 10% in the absence of added cations or in Mn$^{2+}$.

**Latent period, eclipse period and burst size**

The latent period was consistently found to be 25 min, whilst the burst size varied between 50 and 70 p.f.u./cell. The eclipse period was measured in a parallel experiment employing premature lysis to assay for intracellular phage. The number of p.f.u. in the first intracellular phage samples decreased progressively, but then increased very rapidly from 14 min onwards, which is the end of the eclipse period. The small number of p.f.u. detected in the early samples may be due to the processing procedure causing the release of some adsorbed phage particles which had not, at that stage, injected their DNA. Control experiments in which a suspension of phage particles was subjected to the same treatment (freezing and thawing, and osmotic shock) as the premature lysis samples, did not show any effect on the viability of the phage.

**Inhibition of host DNA synthesis**

Synthesis of DNA in parallel infected and uninfected cultures of *S. aureus* was detected by pulse labelling with $^3$H-thymidine. The DNA extracted from uninfected cells sedimented consistently as a single sharp peak with the maximum occurring in fractions 26 or 27 (Fig. 5). With the exception of the sample taken at 5 min post-infection, the infected cell extracts failed to show any incorporation of the pulse of $^3$H-thymidine into material sedimenting in the position of host DNA. Whether or not the small peak of $^3$H material sedimenting in the bacterial DNA position in the 5 min extract was, in fact, in host DNA is uncertain. Even if this was the case, the level of $^3$H incorporated was greatly reduced from that in the comparable uninfected extract. This indicates that inhibition of host DNA synthesis had occurred during the first 5 min and that little, if any, host DNA synthesis was possible during the latent period. In the profiles for the DNAs from the infected bacteria the $^3$H sedimenting in the gradient in the region of fraction 30, and also near the bottom of each gradient, was shown in a separate double-labelling experiment to be in phage DNA. The bacteria were infected with $^{32}$P-labelled phage and then pulse labelled at various times with $^3$H-thymidine. The marker (i.e. $^{32}$P) of the parental phage DNA co-sedimented with the $^3$H used to detect the position of newly synthesized DNA. The latter material sedimenting near the bottom of the gradient (peak in fractions 10 to 12) has been shown to be a replication complex of the phage DNA (Rees, 1979). Because of its sedimentation characteristics we have given it the name ‘rapidly sedimenting complex’ (RSC). The peak of $^3$H material towards the top of the gradient, and which is pronounced in the 24 min sample, contains mature phage DNA (Rees, 1979). The identification of the intracellular replicative forms of K DNA and the details of their structure and properties will be reported in a subsequent paper.
Fig. 5. Effect of K phage infection on host DNA synthesis. Samples (5 ml) of uninfected (○) and infected (●) cultures were taken at the times indicated, immediately pulsed for 30 s with ³H-thymidine (25 μCi/ml) and quickly frozen. The bacteria were subsequently washed, resuspended in TEN and lysed (lysostaphin and Triton X-100). Samples (0.2 ml) of each lysate were sedimented through 5 to 20% sucrose gradients in the SW39 rotor at 75 000 g for 50 min at 4 °C. The radioactivity in 50 μl of each fraction was determined.

Degradation of host DNA

Because infection resulted in inhibition of host DNA synthesis, experiments were performed to determine whether or not this was accompanied by extensive degradation of existing bacterial DNA during the course of phage reproduction. A culture of *S. aureus* was prelabelled with ³H-thymidine and then half was infected with K phage. The DNA extracted from both the infected and the uninfected bacteria at various times post-infection was analysed by sedimentation through sucrose gradients (Fig. 6).

The DNA extracted from the uninfected bacteria gave a consistent sedimentation pattern throughout the duration of the experiment, producing a single peak of ³H-labelled material at fraction 12 or 13. The zero min sample from the infected bacteria gave a sedimentation profile
Staphylococcal bacteriophage K

Fig. 6. Effect of infection on the sedimentation profile of host DNA. The bacterial DNA was labelled before infection by growth in DM2 plus 1 μCi ³H-thymidine/ml. Samples of uninfected (○) or infected (●) cultures were taken at the times indicated and the bacteria separated and lysed by lysostaphin, SDS and Pronase. Each lysate was extracted with phenol and 0.1 ml of the DNA preparations sedimented through sucrose gradients in an SW41 rotor at 150000 g for 3.5 h at 4 °C.

very similar to that of the uninfected extract, but it is noticeable that the amount of tritiated material sedimenting at the top of the gradient was greater in the infected sample. The ³H at the top of the gradient was presumed to be not incorporated into DNA, or incorporated only into short oligodeoxyribonucleotides. By 5 min post-infection, the height of the bacterial DNA peak had begun to decrease and a long shoulder of labelled material, sedimenting more slowly than the host DNA, could be seen; there was also an increase in the amount of 'unincorporated' ³H at the top of the gradient. The bacterial DNA peak was further diminished at 10 min, the majority of the isotope sedimenting as a broad band in a lighter position, and the amount of 'unincorporated' tritium was decreased. In the profile from the 20 min sample the peak of tritiated material corresponding to host DNA was virtually indistinguishable and the amount of 'unincorporated' label had further decreased. At this time the majority of the label on the gradient sedimented as a large peak with a maximum around fraction 20. Control experiments showed that mature phage DNA sedimented with a peak maximum at fraction 20 when centrifuged in identical conditions.

**Utilization of host DNA in the production of phage DNA**

To confirm that nucleotides from host DNA were subsequently incorporated into phage DNA, bacteria prelabelled with ³H-thymidine were infected with K phage. After phage reproduction and lysis had occurred, the phage progeny were isolated by high-speed centrifugation and the ³H content of the various fractions determined. Prior to infection,
Table 2. Distribution of $^3$H after production of K phage in bacteria with DNA labelled with $^3$H-thymidine before infection

<table>
<thead>
<tr>
<th>Host bacteria before infection*</th>
<th>Total $^3$H d/min in fraction</th>
<th>% of total radioactivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>In DNA (TCA precipitate)</td>
<td>2464 164</td>
<td>99.3</td>
</tr>
<tr>
<td>Unincorporated (TCA-soluble)</td>
<td>17 118</td>
<td>0.7</td>
</tr>
<tr>
<td>After infection and lysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In phage pellet</td>
<td>642 073</td>
<td>25.9</td>
</tr>
<tr>
<td>In supernatant after removal of phage</td>
<td>1008 850</td>
<td>43.9</td>
</tr>
<tr>
<td>In TCA precipitate from supernatant</td>
<td>106 909</td>
<td>4.3</td>
</tr>
<tr>
<td>Not precipitated from supernatant by TCA</td>
<td>910 191</td>
<td>36.7</td>
</tr>
</tbody>
</table>

* The bacteria were prelabelled by growth in DM2 plus $^3$H-thymidine (1 μCi/ml), harvested, washed, resuspended in DM1 and 10 min later infected with phage K. The subsequent lysate was clarified (2000 g, 10 min), the phage particles sedimented (90000 g for 1 h at 4 °C), resuspended in TC and clarified. Samples were treated with trichloroacetic acid (TCA), final concentration 5% (w/v) for 15 min at 4 °C, the precipitates collected, washed and counted.

† Results are expressed as a percentage of the total $^3$H in the bacteria before infection.

Table 3. Use of antiserum to precipitate phage produced in bacteria with prelabelled DNA

<table>
<thead>
<tr>
<th>$^3$H distribution site</th>
<th>1:10</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phage–antibody precipitate (P)†</td>
<td>106 895</td>
<td>142 230</td>
<td>144 865</td>
<td>149 685</td>
</tr>
<tr>
<td>Recovery in P relative to initial $^3$H (%)</td>
<td>48.6</td>
<td>64.7</td>
<td>65.9</td>
<td>68.1</td>
</tr>
<tr>
<td>Supernatant (S)†</td>
<td>90 775</td>
<td>53 743</td>
<td>56 879</td>
<td>65 628</td>
</tr>
<tr>
<td>$^3$H not precipitated by antiserum (%)</td>
<td>41.3</td>
<td>24.5</td>
<td>25.9</td>
<td>29.9</td>
</tr>
<tr>
<td>Total recovery (P + S; %)</td>
<td>89.9</td>
<td>89.2</td>
<td>91.8</td>
<td>98.0</td>
</tr>
</tbody>
</table>

* Antiserum (0.16 ml) was added to 1.5 ml phage preparation containing 219 790 d/min and the mixture incubated at 45 °C for 2 h and then at 4 °C overnight. Each precipitate was collected (2000 g for 30 min), resuspended in TC (1 ml) and counted. The neutralization constant of undiluted antiserum was 880 min⁻¹.

† Figures refer to total d/min.

99.3 % of the $^3$H in the host bacteria was precipitated by ice-cold 5% TCA, i.e. it was in intact DNA molecules or long oligonucleotides (Table 2). The conditions used to sediment the phage particles from the lysate should not have sedimented the host DNA. After removal of the phage, treatment of the lysate supernatant with ice-cold TCA revealed that only 9.8 % of the $^3$H label was precipitated and 83.6 % was cold TCA-soluble (recovery relative to total initially present 93.4 %). These results confirmed the finding that during phage reproduction the majority of the host DNA is degraded to short oligonucleotides or nucleotides. Following infection, 69.8 % of the initial bacterial label was recovered in the phage pellet and supernatant. The bacterial debris, collected by low-speed centrifugation of the lysate and from the resuspended phage pellet, contained a further 15.25 % of the $^3$H initially present; the remainder of the $^3$H was probably lost as a result of difficulties in handling the small amounts of pelleted material quantitatively. The resuspended phage pellet contained 25.9 % of the label found in the bacteria before infection, so there is undoubtedly utilization of host DNA nucleotides in the synthesis of phage DNA since immediately before infection the level of $^3$H in the bacteria which was not precipitated by TCA was only 0.7 % of the total.

To prove that the tritium detected in the pellet sedimented by ultracentrifugation was present in phage particles, it was decided to precipitate the phage specifically by treating the preparation with phage antiserum (cf. Maaløe & Watson, 1951). A similar experiment was therefore done on a larger scale to provide sufficient phage suspension to study precipitation at four concentrations of antiserum. Under the conditions used, up to 68 % of the $^3$H initially present was recovered in the precipitate (Table 3). The rest of the label remained in the
supernatant, and failure to precipitate all the tritium could be due to the known fragility of the K phage particle (Wyckoff, 1938; Hotchin, 1954): after exposure to high centrifugal forces and packing of the particles into a pellet, the phage is difficult to resuspend and recovery of viable particles is often less than 50%.

**Kinetics of DNA synthesis in uninfected and infected S. aureus**

Preliminary optimization experiments to determine the duration and cell concentration for pulse labelling were performed on uninfected bacteria. Uptake of $^3$H-thymidine (initially 2 $\mu$Ci/ml) was proportional to pulse duration for up to 2 min and, based on a pulse time of 1 min, the rate of uptake of $^3$H was proportional to bacterial density up to a limit of $4 \times 10^8$ viable bacteria/ml. Accordingly, in all subsequent experiments, a 1 min pulse of $^3$H-thymidine (2 $\mu$Ci/ml) and an initial bacterial concentration of $2 \times 10^8$ viable bacteria/ml were used.

When uninfected bacteria were exposed continuously to $^3$H-thymidine, incorporation of $^3$H into TCA-insoluble material increased linearly with time up to 40 min, before slowing slightly by 50 min and then decreasing (Fig. 7a). The failure to incorporate further label after 50 min is probably due to exhaustion of the supply of labelled thymidine in the medium. The total uptake of $^3$H-thymidine by the infected culture was similar to, but appreciably lower than, that of the uninfected bacteria, e.g. at 30 min it was only 60% of the latter. After 40 min there was little further uptake by the infected culture, since lysis set in at about 30 min. The small
rise in total incorporation after 60 min was probably due to the growth of a small number of surviving bacteria.

The rate of DNA synthesis, measured by pulse labelling, showed that $^3$H uptake into uninfected bacteria taken at progressively later times followed a series of increases interspersed with plateau regions (Fig. 7b). The increases and plateaux appeared to be regularly spaced, one cycle occurring approx. every 20 min. Since the doubling time of the bacteria in the conditions used was also about 20 min it seemed likely that the 15 min incubation in TC prior to dilution into growth medium had resulted in at least partial synchronization of the growth of the uninfected bacteria. The pattern of rate of uptake of $^3$H into infected cells did not show any of the regularity of that found with the uninfected bacteria (Fig. 7b). In the initial stages of infection (the first 15 min) the rates of uptake in the two cultures were similar, but when the uninfected cells entered a plateau at about 14 min the rate of uptake in the infected bacteria continued to increase to a peak until at 20 min it was twice the corresponding rate in the uninfected culture. After 20 min the rate of isotope incorporation by the infected culture decreased a little, but remained more or less constant for a further 10 min and then decreased rapidly. This decrease after 30 min was due to the onset of lysis in the majority of bacteria.

The uptake of $^3$H-thymidine was taken to be a true measure of DNA synthesis because in a separate experiment it was shown that, following a 1 min pulse with 2 $\mu$Ci $^3$H-thymidine/ml, 95% of the label taken up by both infected and uninfected bacteria was incorporated into cold TCA-insoluble material. In view of the previous experiments which have shown that, following infection, host DNA synthesis is inhibited and that degradation and re-utilization of the DNA by the phage also occurs, these experiments show that most, if not all, of the DNA synthesis detected in infected cells is in fact due to the synthesis of phage DNA.

**Discussion**

The results of the study of the morphology of bacteriophage K are in agreement with those of previous workers who have examined the staphylococcal bacteriophages in serological group D (Hotchin, 1954; Lapchine & Enjalbert, 1965): all of these phages have a well-defined head and tail. Hotchin derived his data on the dimensions of phage K from shadowed preparations and found the diam. of the basal structure varied between 25 and 65 nm. Some variability was observed in the present study but the range was not so wide in a given type of stained preparation (i.e. UA or PTA) and the mean value was nearer to the upper end of Hotchin’s measurement (Table 1). The values published by Hotchin (1954) for the K phage head diam. and tail length (82 nm and 238 nm respectively) are greater than those reported here, although this is probably due to the deposition of metal in the shadowing process (Misra & Das Gupta, 1965). With the exception of the phage tail and core diameters, there is good agreement between the dimensions of phage K stained with PTA and those published by Lapchine & Enjalbert (1965) in their study of six serological group D phages (but not phage K) stained in the same way.

When micrographs of a large number of phage particles stained with UA or PTA were measured, the dimensions of the K phage were found to be dependent on the stain employed (Table 1). Bradley (1962) also noticed a difference in the dimensions of the head of a serological group A staphylococcal bacteriophage (phage 70) when stained with UA instead of PTA. To account for this, he proposed that the PTA caused the collapse of the phage head, thereby increasing its apparent diameter, whereas staining with UA resulted in shrinkage of the head. However, the effect appears to be reversed when applied to the phage tail: in those instances where there is a significant difference in the dimensions of the tail structures, the values obtained in PTA-stained preparations are lower than those stained with UA. It is noticeable that staining with PTA caused the tails of the majority of K phage particles to
contract, indicating that the stain causes some conformational change. It is perhaps this induced alteration in tail structure that has resulted in apparent shrinkage of the tail when stained with PTA.

In the determination of the contour length of viral DNA molecules the values obtained here for the T7 DNA (12.0 \( \mu \text{m} \), s.d. ±0.5) were very similar to those (12.5 \( \mu \text{m} \)) reported by Lang (1970), and the s.d. was relatively small at 4% of the mean. In contrast, the s.d. of the mean value obtained for the K phage DNA (16.1 \( \mu \text{m} \), s.d. ±2.6) amounted to 16% of the mean, and leads to the conclusion that the length of this DNA is more variable than that of T7. The apparent heterogeneity could be due to variation in the length of DNA packaged by phage heads during assembly (cf. Freifelder, 1966), a situation found with several other phages such as T4, T1, P22 and Mu. Alternatively, K phage DNA could be more susceptible to systematic errors or variation in the method that do not affect the observed length of T7 DNA. Although the G + C content of K DNA is relatively low (30%), it is of the same order (31 to 32%) as that of a number of strains of \textit{S. aureus} (Silvestri & Hill, 1965). In calculations of the mol. wt. of the K DNA, we used the molar linear density value obtained by Lang (1970) for T7, but this value may be affected by the G + C composition of the DNA (Freifelder, 1970), and the G + C content of T7 is much higher (52%) than that of K phage.

Infection with K phage apparently resulted in the immediate inhibition of host DNA synthesis since no bacterial DNA could be detected by sedimentation of extracts of infected bacteria pulse labelled at zero min post-infection (Fig. 5). A similar inhibition of host DNA synthesis has been noted for \textit{E. coli} infected with bacteriophage T4 (Cohen, 1947) and, in the case of Gram-positive bacteria, for phage-infected \textit{Bacillus subtilis} (Hemphill & Whiteley, 1975). Degradation of the \textit{S. aureus} DNA began within the first 5 min of infection and was revealed as a progressive loss of its normal sedimentation properties and a conversion to small oligonucleotides and nucleotides which, because of their size, remain near the top of the gradient (Fig. 6). At later times in infection the amount of these labelled small mol. wt. materials progressively decreased, presumably because of reincorporation into phage DNA. This view was supported by the appearance at 20 min post-infection of a new peak of tritiated material sedimenting in the same position as mature phage DNA. The transfer of \(^3\text{H}\)-thymidine from host DNA to progeny phage particles during infection (Table 2) confirmed the utilization of host DNA degradation products in the synthesis of phage DNA. Host DNA degradation also occurs in \textit{E. coli} following infection with T4 (Kutter & Wiberg, 1968) or T7 (Sadowski & Kerr, 1970), and begins, respectively, at about 7 or 5 min post-infection, somewhat more slowly than is the case with K phage. Whether K phage specifies its own nucleases for the degradation of host DNA, as does T4 (Wiberg, 1966), is not yet known, nor is it understood how the degradation enzymes are apparently able to act specifically on the host DNA with no effect on the infecting phage DNA, particularly since K phage DNA does not appear to contain any ‘unusual’ bases which may otherwise serve to identify it as different from the host.

Although phage K resembles the T-even numbered phages of \textit{E. coli}, there are important differences. These two types of phages have similar morphology and both have a contractile tail ending in a complicated basal structure. However, the details of the K phage basal tuft are still uncertain: no doubt clearer electron micrographs will be obtained when the individual components of disrupted phage particles can be examined. The overall effect of K phage on host DNA synthesis is the same as that of T-even phages on their host, but in other respects K phage has a less dramatic effect on nucleic acid synthesis in the infected bacteria. Thus, there is here no apparent delay in the onset of viral DNA synthesis. Moreover, whereas RNA synthesis continues though at a reduced level in K phage-infected bacteria (G. Archbold & B. A. Fry, unpublished results), in the T phage-infected bacteria there is no net increase in RNA after infection although phage mRNA is synthesized (Cohen, 1947).
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REFERENCES


Staphylococcal bacteriophage K


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