Inhibitory action of sodium tripolyphosphate (Na-TPP) to P-phages growth was studied. Na-TPP had no phagicidal action, did not prevent adsorption of phage and inhibited formation of infected cells. Furthermore, no phage yield occurred when Na-TPP was added within 5 min of infection, but addition after that time resulted in normal phage growth. DNA synthesis of infected cells was blocked by Na-TPP added early in the latent period (within 5 min), but addition after that time failed to block its synthesis. RNA synthesis of infected cells was not affected even when Na-TPP was added at various times in the latent period. Furthermore, 72 per cent of 32P-labeled phage P-1 adsorbed in the medium containing Na-TPP was separated by the blendor treatment, but only 12 per cent was separated when adsorbed in the absence of Na-TPP. These results indicate that Na-TPP inhibits the injection of phage DNA into the host cell.

In a previous paper, the authors have demonstrated that the growth of P-phages attacking Microbacterium ammoniaphilum, which produces a large amount of L-glutamic acid, is suppressed by the addition of citrate, oxalate, sodium tri- or tetrapolyphosphate to the medium. Namely, such chemical substances have properties of inhibiting phage growth without affecting bacterial growth and L-glutamic acid production. The similar results were already reported by Oki et al. in the industrial L-glutamic acid fermentation employing Brevibacterium lactofermentum No. 2256. They showed that both tetracycline and some surface-active agents, such as polyoxyethylene fatty acid ester or polyoxyethylene alkyl ether, inhibited the intracellular phage growth by suppressing the DNA synthesis and the adsorption of phage onto host cell, respectively. Hershey and Chase successfully used isotope-labeled phage for the demonstration of the injection on phage DNA. They found that 75 to 80 per cent of the labeled T2-phage sulfur could be stripped from the infected cells by a blendor treatment, whereas only 20 to 30 per cent of the labeled phage phosphorous was liberated into the medium, a half of which without any agitation. From these results, they speculated that, after adsorption of phage T2, the phage membrane remains on the bacterial surface, from which it can be stripped by a blendor without affecting the course of infection, and, in contrast, most of the phage DNA enters the host cell soon after adsorption.
after phage adsorption. Luria and Steiner\textsuperscript{5)} also reported that 80 per cent of \textsuperscript{32}P-labeled T5 phage adsorbed in a medium containing no Ca\textsuperscript{2+} was separated by a waring blendor, but only 10 per cent was separated when adsorbed in a medium containing Ca\textsuperscript{2+}, and furthermore, the same results as that obtained in the Ca\textsuperscript{2+} deficient medium were gained by the presence of citric acid, oxalic acid or other Ca\textsuperscript{2+} binding agents.

The purpose of the present paper is to describe that, in this P-phage system, Na-TPP acts as interference with the injection of phage DNA into the host cell.

\textbf{MATERIALS AND METHODS}

\textbf{Phage and Bacterial Strain.} Phage P-\textsuperscript{16)} and its host strain A of \textit{Microbacterium ammoniaphilum} were used throughout the work.

\textbf{Cultural Conditions.} Media and culture conditions were the same as described previously.\textsuperscript{1)}

\textbf{Preparation of \textsuperscript{32}P-labeled phage.} Host strain (final $5 \times 10^9$/ml) and phage P-1 (final $5 \times 10^6$/ml) were inoculated in B-medium described previously.\textsuperscript{1)} They were incubated at 30°C for 10 hr. After cultivation, phages were harvested and washed thrice with B-medium by differential centrifugation at 40,000 rpm and 6000 rpm.

\textbf{Assay procedure.} The phage was assayed by the agar layer method.\textsuperscript{7)} Nucleic acid and protein were fractionated by Schneider’s method.\textsuperscript{8)} RNA, DNA and protein were measured with the orcinol reaction,\textsuperscript{9)} the diphenylamine reaction,\textsuperscript{10)} and the Nessler reagent method,\textsuperscript{11)} respectively. Radioactivity determination was made with use of planchet counting with a GM counter (Kaken model 32).

\textbf{RESULTS}

\textbf{Effect of Na-TPP on Phage Growth}

Effect of Na-TPP on the increase of phages reproduced by infection was studied. The results are shown in Fig. 1. In infected control without Na-TPP, the number of infective centers began to increase after 80 min of infection and finally reached to $10^{10}$/ml. Whereas, regardless of the phage types, the number of infective centers decreased one-sidedly as time elapsed with the addition of Na-TPP.

\textbf{Effect of Na-TPP on Free Phage}

The phagicidal effect was studied by the addition of various concentrations of Na-TPP. As given in Table I, no reduction in titer was shown. Namely, Na-TPP had no direct phagicidal action.

\textbf{Effect of Na-TPP on the Formation of Infected Cells}

The formation of infected cells by the infection of phage P-1 was studied. The results are given in Table II. In infected control

\textsuperscript{8)} W. C. Schneider, \textit{J. Biol. Chem.}, \textbf{161}, 293 (1945).
\textsuperscript{9)} G. Ceriott, \textit{ibid.}, \textbf{214}, 59 (1955).
TABLE I. EFFECT OF Na-TPP ON FREE PHAGE

<table>
<thead>
<tr>
<th>Na-TPP added (mg/ml)</th>
<th>Free phage ($\times 10^7$/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>1.69</td>
</tr>
<tr>
<td>0.5</td>
<td>1.65</td>
</tr>
<tr>
<td>1.0</td>
<td>1.68</td>
</tr>
<tr>
<td>5.0</td>
<td>1.68</td>
</tr>
</tbody>
</table>

Phage P-1 (final $1.7 \times 10^7$/ml) in B-medium, at 30°C for 10 hr.

TABLE II. EFFECT OF Na-TPP ON FORMATION OF INFECTED CELL

<table>
<thead>
<tr>
<th>Na-TPP added (mg/ml)</th>
<th>Phage titer ($\times 10^4$/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-C U-φ I-CL</td>
</tr>
<tr>
<td>None</td>
<td>442  440  0</td>
</tr>
<tr>
<td>1.0</td>
<td>440  437  0</td>
</tr>
</tbody>
</table>

Host strain (final $5 \times 10^9$/ml) and phage P-1 (final $4.5 \times 10^6$/ml) were inoculated in B-medium. Adsorption period was 50 min (absorbed over 90%). Antiserum treatment period was 10 min (neutralized 93% of free phage). Adsorption and antiserum treatment were carried out by the same method as described previously.

Effect of Na-TPP added at Different Stages on Phage Growth

The effect of Na-TPP added later in the latent period was studied. The results are shown in Fig. 2. When Na-TPP was added to the medium within 5 min of incubation time no burst occurred, and the number of infective centers reduced as time elapsed just similarly as when Na-TPP was added at zero time. The addition of Na-TPP after that time brought about the increase of infective centers being due to the maturation and liberation of phages adsorbed by the time. From the results that is mentioned above and no phage growth was occurred when Na-TPP was added after infection had completed, it is obvious that the maturation and liberation are not affected by Na-TPP.

Effect of Na-TPP on Synthesis of Nucleic Acid and Protein in Infected Cells

The effect of Na-TPP on the synthesis of RNA, DNA and protein in infected cells was studied. The results are shown in Fig. 3, 4 and 5. Regardless of the added time of Na-TPP, the net synthesis of RNA was similarly...
little. However, the synthesis of DNA was quite different from it. No addition and delayed addition of Na-TPP (after 10 min of incubation time or more) brought about a three to four times increase in total cellular DNA as compared with early addition of Na-

**FIG. 3. Effect of Na-TPP on Synthesis of RNA.**

Host strain (final 5 x 10⁹/ml) and phage P-1 (final 5 x 10⁹/ml) were inoculated in B-medium. Temp.: 30°C. Na-TPP (final 1 mg/ml) was added at various times.

- ○: No addition
- ●: Added at zero min
- △: Added after 5 min
- ×: Added after 10 min
- □: Added after 40 min

**FIG. 4. Effect of Na-TPP on Synthesis of DNA.**

Same explanation as is used in Fig. 3.

**FIG. 5. Effect of Na-TPP on Synthesis of Protein.**

Same explanation as is used in Fig. 3.

- ○: No addition
- ●: Added at zero min
- △: Added after 5 min
- ×: Added after 10 min
- □: Added after 40 min

TPP. The synthesis of protein was not affected by the added time of Na-TPP. These results suggest that Na-TPP inhibits the intracellular phase growth by suppressing the injection of phage DNA into the host cell.

**Demonstration on Acting Point of Na-TPP with Radio Active Phage**

Acting point of Na-TPP to the inhibition of phage growth was traced by using ³²P-labeled phage. The results are shown in Table III. In the system which no Na-TPP was added, only 12 per cent of radioactive phage was

**TABLE III. ³²P-Labeled Phage Liberated with Blendor Treatment**

<table>
<thead>
<tr>
<th>Na-TPP added (mg/ml)</th>
<th>Time treated (min)</th>
<th>Radioactivity (cpm/ml)</th>
<th>Precipitate</th>
<th>Supernatant</th>
<th>Phage liberated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>28195</td>
<td>3135</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>27573</td>
<td>3762</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>27881</td>
<td>3449</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8753</td>
<td>22572</td>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Host strain (final 5 x 10⁹/ml) and ³²P-labeled phage P-1 (final 5 x 10⁹/ml) were inoculated in B-medium with or without Na-TPP. They were cultured at 30°C for 30 min. After cultivation infected cells were harvested and washed twice with B-medium. They were resuspended in equivalent volume of B-medium and were treated with the waring blendor for 5 min. After the treatment they were centrifuged and radioactivity in the precipitate or supernatant was counted, respectively. cpm: count per minute.
liberated in the supernatant by the blendor treatment, the rest being sedimented with host cells. Whereas, in the system which Na-TPP was added, 72 per cent of radioactive phage was liberated by the same treatment. These results show that, since the injection of phage DNA to the host cell has already completed, the greater part of radioactive phages adsorbed in the absence of Na-TPP are not made free by the blendor treatment, but the radioactive phages adsorbed in the presence of Na-TPP are easily liberated by the same treatment because the injection of phage DNA was inhibited.

Although it is not shown in the results, the adsorption rate of phage P-I reached to 60 per cent and the phage growth in the treatment was negligibly small.

**DISCUSSION**

From the results that, in the presence of Na-TPP, the adsorption of P-phages to host cell is not inhibited at all, no infected cells are formed, DNA synthesis is not activated by phage infection, and $^{32}$P-labeled phage adsorbed is easily liberated by a blendor treatment, it is concluded that acting point of Na-TPP to the repression of phage growth is on the inhibition of phage DNA injection into host cell.

So, the following scheme for the inhibitory mechanism is proposed:

$\text{Na-TPP} \\
\text{Free phage} \rightarrow \text{adsorption} \rightarrow \downarrow \text{penetration} \\
\uparrow \text{Burst} \leftarrow \text{maturation} \leftarrow \text{propagation}$

Moreover, considering that P-phages require Mg$^{2+}$ for their growth,$^{12}$ the most conceivable reason for the inhibition of phage growth by Na-TPP is the elimination of Mg$^{2+}$ which is necessary to phage growth.

**Acknowledgement.** The authors wish to express their thanks to Drs. K. Miyai and Y. Kawano, Managers of Asahi Chemical Industry Co., Ltd., for their support in carrying out this study.

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