THE POTENCY OF NASCENT STREPTOCOCCUS BACTERIOPHAGE B

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The phenomenon of bacterial lysis by a filtrable principle was first observed by Twort and reported by him in 1915. Subsequently, a large number of papers were published by various authors on "bacteriophage," as the lytic principle was designated by d'Herelle. They reported that a specific transmissible bacterial lysin had been found for many bacterial species. The early workers believed that only living bacteria could be attacked by lytic filtrate.

Gratia and Rhodes were the first to report the enhanced potency of bacteriophage in the presence of a growing culture of a sensitive strain. They observed rapid lysis of dead staphylococci if lytic principle and a trace of living sensitive staphylococci were inoculated together into culture medium turbid with dead staphylococci. This observation was confirmed by Twort, who noted, further, that in the presence of living sensitive staphylococci the lytic principle is as specific in its action on dead bacteria as in its action on living bacteria.

Reynals also confirmed the observation that dead staphylococci may be lysed by phage in the presence of living staphylococci. He reported, however, that in similar experiments carried out with Escherichia coli or with Shiga bacilli and their corresponding phages lysis of dead bacteria did not occur.

Wollman and Wollman were the first to observe that in the presence of a growing sensitive strain, phage may lyse not only dead bacteria of sensitive strains, but also resistant living bacteria. Working with staphylococcus phage they observed lysis of re-
sistant staphylococci and also of the closely related Sarcina. They commented on the well defined biologic specificity of these reactions.

Rakieten confirmed the observation of the Wollmans that resistant strains of staphylococci may be lysed by phage in the presence of a sensitive strain. He also commented on the specificity of the reaction.

The nature of the lysozyme which attacks the more refractory bacteria has been discussed in the literature. Wollman and Wollman regarded the secondary lysis of bacteria, in themselves refractory to the factor employed, as due indirectly to the bacteriophage, which as they believe, releases specific diastases from the bacteria undergoing dissolution. Pirie separated an enzyme from normal Escherichia coli cells which removed carbohydrate from homologous heat-killed cells. She was unable to demonstrate such an enzyme in concentrated phage preparations. She interpreted her results as favoring the view of the Wollmans.

On the other hand, Sertic, and also Schuurman presented evidence in favor of the view that a lytic enzyme is secreted by phage, which they regard as living substance. Investigating E. coli phage, Sertic demonstrated a lytic zone free of the transmissible lytic principle surrounding the plaques clarified by phage. The non-transmissible active agent in this zone was considered to be a lysozyme. It could be obtained by glycerol extraction as well as by ultrafiltration of the phage. Sertic's observation was confirmed by Schuurman.

In the literature previous to 1933 all observations recorded on the enhanced potency of bacteriophage in the presence of growing sensitive bacteria were made with staphylococcus phage. In that year the writer observed that in the presence of growing sensitive cells streptococcus bacteriophage would lyse strains which were resistant to the lytic filtrate. The observation occurred during a search for streptococcus phage in sewage. The technique for isolating it was as follows: Streptococci were grown in a series of cultures, the medium for the first culture containing 50 per cent of filtered sewage, and for subsequent cultures containing 10 per cent of filtrate from the preceding
culture of the series. With this technique, evidence of lysis may occur in the third or fourth tube of the series if the sewage contains phage specific for the passage strain.

The idea occurred that the search might be hastened if 10 strains of streptococci were mixed for the serial cultures and if the filtrate were tested for lysis of each strain of the mixture individually. The following experiment was carried out to determine the efficacy of the proposed technique. The point to determine was whether a minute quantity of phage might be rendered inert in a medium in which growth of mixed culture occurred, with resistant bacteria greatly predominating. A minute quantity of streptococcus phage B/563 (1 ml. of the 10⁻⁸ dilution) was added to a tube of broth, which was planted with a drop of mixed culture, the mixture containing equal quantities of culture of 10 strains of streptococci. One of the ten was No. 563, the strain used for the propagation of the phage; the remaining 9 strains were known to be resistant to phage B.

After overnight incubation the tube containing the phage and mixed culture was clear. The lysate was filtered and the filtrate was found to lyse strain 563 in dilutions of 10⁻⁹ and lower, but failed to lyse any one of the other 9 strains. The experiment was repeated, with the same results.

Subsequent investigations, previously reported (1938), showed that phage B/563 filtrate will lyse the streptococcus strains belonging to Lancefield’s precipitin group C. Strains belonging to groups A and E are resistant to the lytic filtrate, but are lysed in the presence of phage and growing sensitive streptococci, with few exceptions to that general rule. On the other hand, streptococci of groups B, D, F, G, H and K are completely resistant to phage B/563.

In an earlier publication (1934) the potent phase of bacteriophage at the time of its formation at the expense of sensitive bacteria was designated “nascent”—a term, which is non-committal as to the nature of the potent principle.¹

¹ Todd used the term “nascent strepto-lysin” to designate a phase of streptococcal hemolysin in which it is capable of combination with serum, at the time of its formation.
EXPERIMENTAL

The experiment recorded in table 1 demonstrates lysis by nascent phage B/563 of streptococcus No. 890 of Lancefield's group A, and Griffith's type 13. It is resistant to B/563 filtrate.

Inoculations were made from overnight cultures into tubes containing beef-infusion broth to which varying quantities of phage had been added, with a control tube for each series containing broth without phage.

The tubes of series I were each inoculated with one loopful of culture 563. Faint turbidity appeared in the broth control after four hours of incubation, whereas in the tubes containing phage, lysis prevented the development of visible growth.

The tubes of series II were inoculated with three drops of culture 890. The turbidity was slight after two hours incubation and increased during the next hour. There was no difference between the turbidity of the control tube and of those containing phage.

The tubes of series III were inoculated with a loopful of culture 563 and three drops of culture 890. In the control tube without phage turbidity developed at about the same rate as in the tubes of series II.

<table>
<thead>
<tr>
<th>SERIES</th>
<th>INOCULUM</th>
<th>TIME</th>
<th>PHAGE DILUTION</th>
<th>BROTH CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10^-1</td>
<td>10^-2</td>
</tr>
<tr>
<td>I</td>
<td>Streptococcus 563, 1 loop</td>
<td>2 hours</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hours</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 hours</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overnight</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>II</td>
<td>Streptococcus 890, 3 drops</td>
<td>2 hours</td>
<td>Like the control</td>
<td>Like the control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hours</td>
<td>Like the control</td>
<td>Like the control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 hours</td>
<td>Like the control</td>
<td>Like the control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overnight</td>
<td>Like the control</td>
<td>Like the control</td>
</tr>
<tr>
<td>III</td>
<td>Same as A &amp; B, together</td>
<td>2 hours</td>
<td>Clear</td>
<td>Faintly turbid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hours</td>
<td>Clear</td>
<td>Faintly turbid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 hours</td>
<td>Clear</td>
<td>Faintly turbid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overnight</td>
<td>Turbid</td>
<td>Clear</td>
</tr>
</tbody>
</table>

* The culture was streaked on blood agar plate.
The tubes of series III to which phage was added showed lysis, the time of clearing varying in the three tubes according to the varying quantities of added phage. The tube containing phage in dilution $10^{-1}$ remained clear during the four hours of observation, but secondary growth (identified as strain 890 by the technique described further on) developed during the night. The tube containing phage dilution $10^{-3}$ was less turbid than the control at the two-hour reading; it had cleared an hour later and remained clear overnight. At two hours the tube containing phage dilution $10^{-5}$ was slightly turbid, like the control; at three hours lysis had begun; and at four hours the liquid was clear.

The following evidence indicated that at the two-hour reading the growth in the third tube of series III, inoculated with one loop of strain 563 and three drops of strain 890, was largely made up of cells of strain 890, subsequently lysed: (1) The inoculum with strain 563 was so weak that in the control tube faint turbidity did not appear until two hours later. (2) The turbidity was identical with that of a broth control tube planted at the same time with a similar inoculum of 890 alone. (3) A loopful of the culture after two hours incubation was streaked on a blood agar plate. After incubation 28 isolated colonies were transferred to tubes of broth. In two of the 28 tubes no turbidity developed; in five tubes cultures of strain 563 grew; and in 21 tubes cultures of strain 890 developed, as determined by the following technique: The broth cultures were streaked on blood agar plates, together with cultures of 563 and 890 for comparison. The growth of these two strains is readily distinguishable on blood-agar plate, for the massed colonies of strain 563 make a markedly darker, more opaque streak than those of strain 890.

In the preceding experiment the technique for the demonstration of sensitivity to nascent phage described in a previous publication was used. The results gave convincing evidence that strain 890 may be lysed by nascent phage B/563. In the following experiment using a simpler technique, the lysis of cells of strain 890 by nascent phage B/563 was demonstrated by unequivocal results.

To each of five tubes containing 9 ml. of broth 1 ml. of over-
night culture 890 was added, giving a slight but definite turbidity. The tubes were incubated for half an hour, then one tube was heated to kill the bacteria, as a turbidity control. The remaining tubes were treated as described in the protocol in table 2. Lysis of the slightly turbid culture 890 occurred within one and one-half hours after the addition of 1 ml. of phage B/563 and one drop of culture 563.

The enhanced activity of nascent phage may also be demonstrated in plate culture, as shown in the accompanying photograph.

**TABLE 2**

*Lysis of *Streptococcus* 890 by nascent phage B/563*

<table>
<thead>
<tr>
<th>TUBE</th>
<th>ADDITIONS TO SLIGHTLY TURBID CULTURE 890</th>
<th>READING AFTER 1½ HOURS' INCUBATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Increased turbidity</td>
</tr>
<tr>
<td>2</td>
<td>1 ml. phage</td>
<td>Turbidity as in tube 1</td>
</tr>
<tr>
<td>3</td>
<td>1 drop culture 563</td>
<td>Turbidity as in tube 1</td>
</tr>
<tr>
<td>4</td>
<td>1 ml. phage; 1 drop culture 563</td>
<td>Clear</td>
</tr>
</tbody>
</table>

Six circular areas were marked off on a plate containing beef-infusion glucose agar. A loopful of culture was smeared over each area, as follows:

1. Culture 890.
2 and 6. Culture 563.
3. Cultures 563 and 890, mixed in the proportion of 1:10.
4. Cultures 563 and 890, mixed in the proportion of 1:40.
5. Cultures 563 and 890, mixed in the proportion of 1:100.

The plate was incubated for one hour, then a small droplet of undiluted phage B/563 filtrate was placed by means of a needle in the center of each area excepting No. 2. The plate was then incubated for 12 hours. In area 1, inoculated with strain 890, with phage added later, and in area 2, inoculated with strain 563 without the addition of phage, no lysis occurred. In area 3, inoculated with strains 563 and 890 in the proportion of 1:10, and in area 6, inoculated with strain 563 alone, a central area was cleared by the droplet of phage; in areas 4 and 5 inoculated with a mixture
of strains 563 and 890 in the proportions of 1:40 and 1:100, respectively, a clear circle surrounded a central disk of unlysed colonies. On several plates planted in the same way on different dates, the clear ring always appeared in the area planted with the mixture of 563 and 890 in the proportion of 1:100; not always in the area planted with the mixture in the proportion of 1:40, which sometimes showed a clear central disk, as in areas 3 and 6.

SUMMARY

Experiments are described which demonstrate the enhanced potency of streptococcus bacteriophage in the nascent stage.
Strain 890, of Lancefield's group A, which is resistant to phage B/563 filtrate, is shown to be sensitive to the lytic principle in the presence of a growing culture of the sensitive strain 563.

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