TECHNIQUE FOR THE DETERMINATION OF THE SENSITIVITY OF
A STRAIN OF STREPTOCOCCUS TO BACTERIOPHAGE
OF TYPE A, B, C OR D

ALICE C. EVANS

National Institute of Health, Bethesda, Maryland

Received for publication October 13, 1941

In a previous publication (1934) four serologic types\(^1\) of streptococcal bacteriophage were described and designated A, B, C and D, to conform with the nomenclature of phage specific to other genera of bacteria.

It has been shown (Evans) that phage in the nascent state, i.e., in the presence of cells of a sensitive strain undergoing lysis, is more potent than in the filtered state. The nascent and also the filtered phage may be utilized for the differentiation of certain streptococcic groups for which one or another of the several types of phage have special affinities. Phage A is an exception, however, in that it has not been found useful in classification studies because in the nascent state it is capable of lysing more or less completely all strains of Lancefield's Group A; the filtered phage \(A_{751}\) may or may not attack the strains of the various subgroups of Group A, irrespective of other characters.

Phage B is specific in its reactions and may be utilized to identify the streptococci of Lancefield's Group C, which are the only ones sensitive to filtered phage B. Although the strains of Groups A and E are resistant to filtered phage B, they are sensitive to it in the nascent state, with few exceptions to that general rule. Streptococci of the other Lancefield groups are completely resistant to Phage B (Evans and Verder, 1938).

Phage C has a special affinity for the strains of *Streptococcus scarlatinae* (unpublished data) with which it gives clear lysis in high dilution of filtered phage. Filtered phage C lysed many of the strains of other streptococcic species of group A, but with notably less vigor than it lysed the strains of *S. scarlatinae*. The strains of the epidemicus group are characterized by resistance to phage C (Evans, 1940).

Filtered phage D has a special affinity for the enterococci, effecting complete lysis in a wide range of dilutions, whereas only an occasional strain of Group A is slightly sensitive to filtered phage D. Many strains of group A are slightly sensitive to phage D in the nascent state, but correlation of this with other characters of group A has not been observed (unpublished data).

\(^1\) In this series of publications "race" refers to a pure phage of any isolation, being analogous to the use of the term "strain" in bacteriologic literature; "type" refers to the serologic identity of the race as determined by neutralization by specific serum.
The phages used in our studies are designated A\textsubscript{751}, B\textsubscript{562}, C\textsubscript{594} and D\textsubscript{652}, the figure referring to the strain of streptococcus which served for the propagation of the phage.

The technique for determining the sensitivity of a given strain of streptococcus to phage as previously described (Evans, 1936) has been modified and is therefore described here again in detail. This modified technique may be used to determine the sensitivity of any strain of streptococcus to phage A, B, C or D.

The test for sensitivity of a strain of streptococcus to one of the types of phage can be most clearly described by an example. The testing of strain 1501 for sensitivity to phage C\textsubscript{594} requires 11 tubes, each containing 4.5 ml. of neopeptone broth. To each of 9 of the tubes of broth (3 sets of 3 tubes each) is added 0.5 ml. of filtered phage in such dilution that the final dilutions are 10\textsuperscript{-1}, 10\textsuperscript{-3} and 10\textsuperscript{-4}.

Overnight culture is used for the inoculum. It is diluted by transferring a loopful to a tube containing 4.5 ml. of broth. The inoculum is one drop of diluted culture. An example of a protocol is given in table 1 which shows the

| TABLE 1 |
| Examples of a test for sensitivity of a strain of streptococcus to phage C\textsubscript{594} |

<table>
<thead>
<tr>
<th>STREPTOCOCCIC INOCULUM</th>
<th>APPEARANCE OF BROTH</th>
<th>DILUTED PHAGE FILTRATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10\textsuperscript{-1}</td>
</tr>
<tr>
<td>Strain 594</td>
<td>Turbid</td>
<td>4</td>
</tr>
<tr>
<td>Strain 1501</td>
<td>Turbid</td>
<td>0</td>
</tr>
<tr>
<td>Strains 594 and 1501</td>
<td>Turbid</td>
<td>0</td>
</tr>
</tbody>
</table>

0 designates turbidity as in the control broth culture.
1, 2, and 3 designate decreasing degrees of turbidity.
4 designates complete lysis.

data for the test to determine the sensitivity of Strain 1501 to phage C\textsubscript{594}. The tubes with diluted phage of one set and a tube of broth are inoculated with Strain 594. These tubes act as a control test to determine the activity of the sample of phage and the viability and sensitivity of Strain 594. Clearing should occur in all three tubes containing phage. The tubes of diluted phage of another set and a tube of broth are inoculated with Strain 1501. The tubes with diluted phage of the third set are inoculated with both strains, 1501 and 594. The cultures are incubated about 18 hours, and then readings are made for lysis. The method of making the readings is illustrated in table 1 which shows that Strain 594 (of the species of \textit{S. scarlatinae}) is highly sensitive to phage C; Strain 1501 is resistant to the filtered phage, but it is lysed by phage C in the nascent state, under certain conditions such as those which prevailed in the second and third tubes of the series.

The enhanced activity of nascent phage was the subject of a previous publication (Evans, 1940). It is illustrated by the sensitivity of Strain 1501 to phage C\textsubscript{594} at the moment of its development, although it is resistant to the filtered phage.
SENSITIVITY OF STREPTOCOCCUS TO BACTERIOPHAGE

The purpose of testing with three dilutions of phage, varying from low to high, is to provide a wide range of conditions for the unknown vagaries of sensitivity of the strain in question, because zoning of the reaction as illustrated in table 1 is common. In the case of any race of streptococcic phage, lysis by the filtered or nascent phage may occur in the higher when it is not evident in the lower dilution, or, lysis may occur in the lower but not in the higher dilutions containing a weaker concentration of phage.

SUMMARY

The technique for the determination of sensitivity of a strain of streptococcus to bacteriophage is described. The same technique is used for the determination of the sensitivity of any strain of streptococcus to any of the types of streptococcic phage A, B, C or D.

REFERENCES

Evans, Alice C. 1940 The potency of nascent streptococcus bacteriophage B. J. Bact., 39, 597-604.
Evans, Alice C. 1940b Studies on hemolytic streptococci. VI. The epidemicus group. J. Bact., 40, 215-222.
Evans, Alice C., and Verder, Elizabeth 1938 Studies on hemolytic streptococci. V. The characteristics of human and animal strains of groups A and C. J. Bact., 36, 133-147.