ANOTHER SEROLOGIC TYPE OF STREPTOCOCCIC
BACTERIOPHAGE

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In a study of a group of streptococci of Lancefield’s Group A, biochemical
characters, including fermentative reactions and sensitivity to bacteriophage,
were investigated. It was observed that the strains of two of the sub-groups:
(1) the lactose-deficient group (Evans, 1941), and (2) the mannitol-fermenting
group were not particularly sensitive to any of the 4 known types of strepto-
coccic phage. It appeared likely that one or more new types of phage might
be found if a search were made, using strains of streptococci of these two groups
for the substrata.

EXPERIMENTAL

The methods used for the isolation of phage from sewage, and the techniques
for the study of its characters, followed, with certain modifications, those
previously described (Evans, 1934).

Isolation. Samples of sewage collected for another purpose in February and
March, 1940, from the final clean-out trap leading from hospitals to the city
sewage system were studied. Samples from Baltimore, Boston, New York
City, Evansville, Indiana, and Washington, D. C. were examined for phage
which might be active against any of six selected strains of streptococci repre-
senting the two mentioned sub-groups.

The sewage was treated in such a manner as to propagate any phage particles
with an affinity for the selected strains which might be present. It was used
as one of the ingredients of a medium in which the streptococci were grown in
serial cultures.

The medium consisted of meat-infusion broth of double strength distributed
in test tubes in 8 ml. amounts and sterilized by heat. Previous to the inocula-
tion, 8 ml. of sewage sterilized by filtration was added to each tube and 4 ml.
of a sterile filtrate was also added. The filtrate will be described presently.

The inoculum for the several culture generations of streptococci in the sewage
medium was in every instance a mixture of the six strains. The mixture was
prepared by adding one drop of overnight broth culture of each strain to 9 ml.
of broth. One drop of this diluted mixed culture was planted in the sewage
medium. After growth overnight the culture in sewage medium was filtered,
and this filtrate was tested to demonstrate any possible lytic activity by planting
each of the six strains in a tube of broth containing 10 per cent of the filtrate.

The 4 ml. of filtrate added to the sewage medium for the first of the series of
mixed streptococcus cultures was from a broth culture inoculated with the
unfiltered sewage and incubated overnight at 37°C. For the second and follow-
ing culture generations of streptococcus the 4 ml. of filtrate added to the medium was from the preceding culture of the series. The process was continued until six or more serial plantings had been made. The filtrate from each of the serial cultures was tested with each of the six strains of streptococcus for evidence of lysis.

A lytic agent active against the mannitol-fermenting strain No. 985 was obtained from two of the sewage samples; after the first enrichment passage in the medium containing Baltimore sewage, and after the third enrichment passage in medium containing the Evansville sewage. No lytic agent was found capable of attacking the other five strains included in the experiment.

Strain 985 was received from Dr. Griffith in 1935, labelled "Beatty, type 17." It was found to be serologically distinct from all other type strains of Griffith, thus agreeing with the results reported by Griffith, and also by Keogh, Simmons and Wilson.

After several serial passages of the new races of phage in culture with strain 985, the filtrates of each race contained the lytic agent in dilutions as high as $10^{-4}$.

The new races of phage were tested for neutralization by antiserum specific to phages of the serologic types A, B, C or D, using a technique previously described (Evans, 1934). Neither of the new races was neutralized by any one of the antiserums. This showed that the new races did not belong to any of the known serologic types.

An antiserum was then prepared by injecting a rabbit with several doses of the filtered Evansville phage according to the technique previously described. The antiserum from this rabbit neutralized the new Baltimore race of phage as well as the Evansville race, but it neutralized none of the phages of types A, B, C or D. These results showed that the two new races belonged to the same type, which differed serologically from types A, B, C and D. The new serologic type was designated E.

**DESCRIPTION OF PHAGE E**

Phage E differs from the four previously known serologic types of streptococcus phages A, B, C and D in its unusual resistance to heat, and in the rapidity of development of secondary cultures.

**Thermolability.** The temperature of inactivation of a freshly prepared sample of phage E985, was determined in triplicate tests. The titer was $10^{-4}$; the pH was 7.4. One-half-ml. quantities were sealed in 5 mm. tubes and immersed for one hour in a water-bath at a constant temperature. Each cooled sample was then added to 4.5 ml. of neopeptone broth which was inoculated with one drop of homologous culture. At the same time a control tube containing broth was inoculated with the culture. The tubes were incubated and read frequently for lysis. If no lysis occurred the culture was filtered and the filtrate was tested for active phage. This process was continued through 3 serial passages in broth culture, after which it was concluded that inactivation was complete if no evidence of lysis was observed.

Phage E985 survived temperatures up to 74–75°C. but after heating at 75–76°C.
no lysis occurred. The temperature of inactivation was thus shown to be higher by 10 degrees for phage E than for any other known streptococcic phage. It had previously been shown that phages A\textsubscript{831} and B\textsubscript{86} were inactivated at 60°C; phage C\textsubscript{654} was inactivated at 65°C. and phage D was inactivated at 63°C. A test to determine the thermolability of one of the previously described phages, made simultaneously with a test on phage E\textsubscript{985}, confirmed the earlier observation.

Secondary cultures. The development of secondary growth in cultures lysed by phage E\textsubscript{985} was rapid, appearing in 2 to 3 hours after complete lysis. On account of the rapidity of the development of secondary growth it was necessary to modify the technique described for determining sensitivity to phage A, B, C or D (Evans, 1942). Instead of planting the tubes containing phage E with a drop of diluted culture they were planted with a drop of undiluted overnight culture. Incubation was for 6 hours, after which the tubes were placed in a cold room at about 15°C. and readings were made the following day.

The plaques formed by phage E on 1.25 per cent agar containing 0.5 per cent glucose, with streptococcus 985 as the substratum, ranged between 0.5 and 0.75 mm. in diameter. The edges were blurred and irregular. As in broth cultures, secondary growth developed rapidly.

**Range of activity.** The strains of Lancefield's Group A were generally lysed more or less completely by nascent phage E\textsubscript{985} with only an occasional resistant strain found. Hence, sensitivity to nascent phage E\textsubscript{985} was found to be a character of no value for the differentiation of streptococci. Filtered phage E\textsubscript{985} lysed the homologous strain in dilutions as high as 10\textsuperscript{-4}. Of the 295 heterologous strains of Lancefield's Group A tested for sensitivity to filtered phage E\textsubscript{985}, 54 were found to be more or less sensitive. Of the 295 strains 40 fermented mannitol and 255 did not. That there is a correlation between mannitol fermentation and sensitivity to phage E\textsubscript{985} is indicated in table 1 which shows that the frequency of sensitivity to phage E\textsubscript{985} in mannitol-positive strains was more than four times that in mannitol-negative strains.

Of the 54 strains which showed more or less sensitivity to phage E\textsubscript{985}, none but the homologous strain was lysed completely in all 3 tubes of the test. The unique high degree of sensitivity of strain 985 to phage E is in agreement with its unique agglutinogenic property. Of the 295 strains tested for sensitivity to phage E\textsubscript{985}, 163 were also tested for agglutinability by an antiserum prepared by treating a rabbit with a course of injections with strain 985. Only the

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**TABLE 1**

*Correlation between the characters of mannitol fermentation and sensitivity to phage E\textsubscript{985}*

<table>
<thead>
<tr>
<th>MANNITOL FERMENTATION</th>
<th>SENSITIVITY TO FILTERED PHAGE E\textsubscript{985}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
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<tr>
<td>-</td>
<td>33</td>
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homologous strain was agglutinated by the serum after absorption to remove non-specific agglutinins according to Griffith's technique. Since it has been found that in streptococci of other serologic types sensitivity to phage is correlated with serologic grouping it is to be expected that when another streptococcus of type 17 is encountered it will be found to be sensitive to phage E985.

SUMMARY

Two races of streptococcic bacteriophage, serologically alike and distinct from the 4 previously known serologic types A, B, C and D, were recovered from sewage.

The new type is designated E. Among 295 strains of streptococci tested, only one was found to be highly sensitive to the new race of phage. This strain, No. 985 of Griffith's type 17, is serologically distinct from 163 strains of streptococcus which were tested for agglutinability in antiserum 985.

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