THE PROTECTIVE EFFECT OF SODIUM CHLORIDE AGAINST THERMAL DESTRUCTION OF THE PHAGE-FORMING MECHANISM IN STAPHYLOCOCCI

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(Received for publication, February 13, 1948)

It was shown several years ago that staphylococci which had been “activated” by rapid growth under favorable conditions and subsequently reduced to a resting state could produce an immediate six- to tenfold rise in [phage] when added to a phage-containing solution (1, 2). The basic experiment for demonstrating the phenomenon was performed by:

1. Growing the staphylococci in oxygenated broth at 36°C. for 2 hours.
2. Washing the cells and resuspending them in Locke’s solution.
3. Maintaining the suspension at 5°C. for 2 hours.
4. Adding 4 ml. of the cell suspension to 1 ml. of phage diluted in Locke’s solution to a titre of $1 \times 10^8$ activity units/ml.
5. Titrating various dilutions of the cell-phage mixture by the activity assay method (3).

The characteristic increase in [phage] occurred within 0.1 hour after adding the cells to the phage. This phage-augmenting property of staphylococci having a history of rapid growth in a favorable medium was not present in normal viable cells harvested 16 to 18 hours after inoculation of an agar substrate. As a working hypothesis it was postulated that the reaction between “activated” bacteria and phage involved some sort of a precursor which upon contact with phage was autocatalytically transformed into phage.

The capacity of “activated” organisms to increase [phage] activity is abolished by concentrations of iodoacetate and methylene blue which leave the cells viable and reproductive. This is true also for exposure to heat and to sonic waves.

In the present paper we wish to report experiments in which the rapid destruction of phage “precursor” by heat was considerably retarded by the presence of sodium chloride.

Experimental Methods

The experiments consisted of exposing “activated” and normal bacteria suspended either in sterile distilled water or in 1 M NaCl solution to a temperature of 44°C. Following such thermal treatment the suspensions were chilled in an ice-water bath, and phage was then added to yield an initial concentration of $2 \times 10^8$ activity units per ml. These suspensions were again iced for an additional 30 minutes to permit inter-
action of "precursor" and added phage before preparing dilutions for activity assay. To determine the proportion of surviving bacteria following heat treatment, viable counts were performed at the beginning and at the end of the experiment, using suspensions which did not contain phage.

"Activated" staphylococci were prepared by growing an 18 hour culture of *Staphylococcus aureus* (the K strain) in a flask of tryptose phosphate broth. Continuous agitation of the medium at 36°C. was accomplished by means of a mechanical shaker mounted in a water bath. "Activation" was usually complete after 1½ hours of growth, at which time the suspension attained a concentration of approximately $1 \times 10^9$ bacteria per ml. Subsequent to "activation" the suspensions were centrifuged and the deposits resuspended in either sterile distilled water or in 1 M NaCl solution and adjusted to a density of $5 \times 10^8$ bacteria per ml. Normal "non-activated" bacterial suspensions were prepared in a similar manner except that the period of rapid growth was omitted. The "activated" and normal suspensions were then placed in a 44°C. water bath and 4 ml. samples were removed as soon as they had attained 44°C.; further 4 ml. samples were removed after 15 minutes at this temperature. As controls, 4 ml. samples were also taken prior to thermal treatment. All samples were chilled in ice-water bath before adding 1 ml. of phage (the K phage) containing $1 \times 10^9$ activity units per ml. Immediately after the addition of phage the samples were placed in an ice-water bath for 30 minutes; dilutions were then prepared for activity titration according to the method of Krueger. The total number of bacteria present in the preparations was determined by means of a Klett-Summerson photoelectric colorimeter which had previously been calibrated with cell suspensions of known density. Viable counts were determined by plating proper dilutions in tryptose agar; counts were made after incubation at 36°C. for 48 hours.

**DISCUSSION OF EXPERIMENTAL RESULTS**

Table I illustrates the results obtained in three individual experiments. Normal "non-activated" staphylococci which are suspended in water and exposed to a temperature of 44°C. for 15 minutes are no different from bacteria which are not subjected to thermal treatment. This is evidenced by the fact that there is no significant change in the amount of phage present when the cells are added to phage-containing solutions, as a test for "precursor," and by the fact that the number of surviving bacteria shows only a slight decrease.

In marked contrast is the behavior of "activated" bacteria suspended in water. After 15 minutes at 44°C. there is a very noticeable drop in the number of phage activity units, the loss ranging from 15- to 71-fold; concomitantly, there is a considerable reduction in the number of viable staphylococci, the approximate number of survivors being only 1 to 3.5 per cent of the original concentration. Microscopic examination of the bacteria suspended in sodium chloride solution shows no aggregation of the staphylococci, indicating that this is not the factor responsible for the drop in plate count.

Normal "non-activated" staphylococci, when suspended in 1 M NaCl solution, show practically no change either in phage formed or in the number of
viable bacteria remaining. However, "activated" staphylococci, under these conditions are able to maintain their state of activation without significant reduction in the number of surviving staphylococci.

Apparently 1 m NaCl effectively protects the "precursor" content of "activated" bacteria against thermal inactivation and also preserves the viability of the cells. Various electrolyte effects on the phage-bacterium reaction have been observed previously. Thus, Krueger and West (4) found that minute concentrations of Mn++ accelerated bacteriophagy by lowering the ratio of phage to bacteria requisite for massive lysis. Scribner and Krueger (5) observed that the addition of 0.25 m NaCl raised the lytic threshold five- to ten-fold and thereby increased the end titre of the lysate. Krueger and Strietmann (6) noted similar results in studies on the phage-bacterium reaction conducted in the presence of Na$_2$SO$_4$.

**SUMMARY AND CONCLUSIONS**

Staphylococci which have been allowed to grow rapidly in a favorable environment and subsequently have been maintained in a resting state character-

<table>
<thead>
<tr>
<th>Suspension</th>
<th>Diluent</th>
<th>Exposure</th>
<th>Activity units/ml</th>
<th>Viable bacteria/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-activated</td>
<td>Water</td>
<td>Unheated</td>
<td>$\times 10^4$</td>
<td>$\times 10^4$</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>1.0</td>
<td>0.56</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15</td>
<td>1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>&quot;Activated&quot;</td>
<td>&quot;</td>
<td>Unheated</td>
<td>7.0</td>
<td>32.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>7.0</td>
<td>32.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15</td>
<td>0.48</td>
<td>0.45</td>
</tr>
<tr>
<td>Non-activated</td>
<td>NaCl</td>
<td>Unheated</td>
<td>1.0</td>
<td>0.56</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>1.0</td>
<td>0.56</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15</td>
<td>1.0</td>
<td>0.17</td>
</tr>
<tr>
<td>&quot;Activated&quot;</td>
<td>&quot;</td>
<td>Unheated</td>
<td>7.0</td>
<td>32.0</td>
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<tr>
<td>&quot;</td>
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<td>0</td>
<td>7.0</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>15</td>
<td>5.6</td>
<td>22.0</td>
</tr>
</tbody>
</table>

The symbol — signifies that the sample was not tested.

The initial phage concentration in all samples was $2.0 \times 10^8$ activity units/ml.
istically produce a sharp rise in [phage] activity titre when added to phage. This capacity is quickly lost when the cells are suspended in distilled water and are exposed to 44 ° C. for a period of 15 minutes; at the same time the viable count drops to approximately 1 to 3.5 per cent of the initial value. 1 m NaCl protects "activated" cells against thermal destruction and preserves the phage-augmenting property. "Non-activated" staphylococci in distilled water suspension do not show this thermolability.

The writer wishes to express his appreciation to Dr. A. P. Krueger for his continued interest in the problem.

BIBLIOGRAPHY