EFFECTS OF SPECIFIC ANTISERA ON THE GROWTH OF BACTERIAL VIRUSES (BACTERIOPHAGES)\textsuperscript{1}

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When a suspension of bacterial viruses is mixed with specific antiserum, the virus particles are rapidly "inactivated," that is, they lose the power to attack bacteria and to multiply. This inactivation is due to the union of the virus particles with the antibody. The union is irreversible but does not destroy the virus. The original virus may be recovered if the antibody is digested with papain (Kalmanson and Bronfenbrenner, 1943). Why does the union of the virus with the antibody inactivate the virus? Does it merely prevent adsorption of the virus on the bacterial host cell or does it block a later step in the process of virus growth? Hershey (private communication) has found that virus which is barely inactivated (by exposure to antiserum for a short time or at a high dilution) is still adsorbed by the bacteria. Under these conditions the antibody must interfere with a later step of virus growth.

In the present paper we wish to report experiments which attack these problems by somewhat different methods. In previous work (Burnet, Keogh, and Lush, 1937; Hershey, 1943; Hershey, Kalmanson, and Bronfenbrenner, 1943) the serum effects were tested by determining differences in the number or size of plaques formed by a virus suspension after various treatments. In our experiments we determined also the latent period of virus growth and the yield of virus from infected bacteria. In the previous study, moreover, treatment of the virus suspension with antiserum always preceded the adsorption of virus on its host. We were interested to find out whether any effect of the antibody on the virus could be detected if the antiserum was added to the virus already adsorbed to bacteria.

In this paper we also wish to report experiments with antibacterial sera. Such sera are always totally inactive against viruses which can attack the bacteria, that is, when antibacterial serum is mixed with virus and incubated, subsequent titrations of the virus show no reduction of the plaque count. However, very striking effects are obtained when the bacteria are treated with antibacterial serum prior to the addition of the virus. The virus growth may then be completely or partially inhibited.

These experiments were begun for the purpose of using the antisera in a study of interference between different viruses simultaneously attacking the same host. The preliminary interference experiments will be reported in a subsequent paper. The preliminary experiments constitute, however, an independent attack on the general problem of the mechanism of growth of bacterial viruses. Our experi-

\textsuperscript{1} Aided by grants from the Rockefeller Foundation and the John and Mary R. Markle Foundation.
ments in this direction are merely exploratory and could be extended further in several ways.

PREPARATION OF THE ANTISERA

Rabbit antisera against viruses\(^1\) gamma and delta were prepared by subcutaneous injections of high titer, filtered broth lysates of these strains. After two courses\(^2\) of injections sera were obtained which inactivated virus measurably in 30 minutes at 37 C at a dilution of 10,000. With virus alpha this procedure yielded an antiserum the titer of which was about ten times lower than those against gamma and delta. A third course of intradermal injections yielded a titer nearly as high as those for the other viruses. All three antisera were strictly specific, that is, each serum was totally inactive against the heterologous strains. They did, however, contain some antibacterial antibody, because of the impurity of the antigens used. This antibacterial activity could be absorbed with suspensions of the host bacteria without reducing the antiviral titer.

The antibacterial serum was obtained by two series of intravenous injections with live washed bacteria. Complete agglutination was effected by a dilution of 1:640 of the serum, and agglutination was noticeable at a dilution of 1:1,280. It was totally inactive against each of the viruses.\(^3\)

INACTIVATION OF FREE VIRUS BY ANTIVIRUS SERUM

The inactivation of bacterial viruses by specific antisera has been studied repeatedly, most recently and in great detail by Kalmanson et al. (1942), Hershey (1943), and Hershey et al. (1943). The virus particles are inactivated by irreversible combination with antibody molecules. One virus particle can combine with several thousand antibody molecules.\(^4\) Inactivation occurs, however, at an early stage of the reaction. Inactivation depends on the combination of the antibody at very specific sites on the virus. One or a very few antibody molecules attaching to these specific sites inactivate the virus. Therefore, the fraction of active virus in a mixture of virus and antiserum decreases exponentially with time. The rate of inactivation is proportional to the concentration of

\(^1\) We are indebted to Dr. A. D. Hershey for the gift of a high titer antigamma serum, to Dr. E. W. Goodpasture for placing the facilities of the Department of Pathology, Vanderbilt Medical School, at our disposal for making antisera, and to Dr. John Buddingh for generous help and advice in the preparation and the testing of the sera.

\(^2\) For a description of the viruses see table 1 of the preceding paper.

\(^3\) Each course consisted of four or five injections of 5 ml of filtered lysates containing about \(5 \times 10^9\) virus particles per ml. The injections were given twice a week, and there was an interval of about one month between courses.

\(^4\) Our first antibacterial serum inactivated virus alpha to a fairly high titer. The cause of this antialpha activity is obscure. The serum was not used in any of the experiments reported in this paper.

\(^5\) Cf. table 7, page 293, of Hershey et al. (1943). The last column of this table is based on an erroneous estimate of the size of the virus particle. Electron microscope pictures (Luria et al., 1943) and a redetermination of the diffusion rate (Hershey, private communication) have established beyond doubt that the “lytic unit” represents one virus particle.
serum. The fraction of active virus may thus be represented by the following formula:

\[ \frac{V}{V_0} = e^{-kt/D} \]

in which \( V_0 \) = concentration of active virus at time 0
\( V \) = concentration of active virus at time \( t \)
\( D \) = dilution of antiserum
\( k \) = specific inactivation constant
\( t \) = time in minutes

![Diagram](image.png)

**Fig. 1.** Inactivation of virus alpha by specific antiserum as function of time times concentration of serum. Three different dilutions of the antiserum, namely, 100, 500, and 2,000. The law expressed by formula (1) would give a straight line in this diagram with a slope independent of the serum dilution.

The "titer" of the serum is measured by \( k \). Given \( k \), the dilution and time of action required for any amount of inactivation can be calculated. For instance, one of our antigamma sera had a titer \( k = 300/\text{min} \). Suppose 99 per cent inactivation in 3 minutes was required. Substitution in (1) tells us that the required serum dilution should be 200.

Inactivation of virus alpha does not follow this simple pattern (figure 1). The rate of inactivation is not constant but decreases as inactivation progresses. When fresh virus is added after inactivation has progressed for some time, the newly added virus is inactivated at the original rate. The retardation of inac-
activation is therefore not due to exhaustion of the antibody molecules in the serum. It must be due to an inhomogeneity among the virus particles. The inhomogeneity may be inherent in the virus suspension or, more likely, it may develop as inactivation progresses. One may assume that virus particles which have combined with a certain number of antibody molecules thereby are less likely to combine with antibody molecules at the critical site (cf. Delbrück and Luria, 1942, p. 20). The rate of inactivation of alpha virus is also not proportional to the concentration of antiserum. It increases more slowly when this concentration is increased. This phenomenon may be explained by assuming the occurrence of a reversible aggregation of the constituents of the antiserum, such that, as the antiserum is diluted, the antibody molecules disaggregate and a greater proportion of them are free to combine with virus. However these peculiarities may be explained, they do not interfere with the use of the antiserum in growth experiments.

**ACTION OF ANTISERUM ON BOUND VIRUS**

Free virus particles combine rapidly and irreversibly with the antibody. The virus is thereby inactivated. Can the virus also be inactivated by combination with the antibody after the virus has been adsorbed on the host, or after it has entered the host? Can the newly grown virus be inactivated before it is liberated from the host? The following experiments answer these questions in the negative.

*General procedure.* One step growth experiments (Delbrück and Luria, 1942) with antiserum added after adsorption, and diluted before lysis beyond the range of action of the antiserum, were conducted.

*Experiment 1.* Single infection with gamma, adsorption for 5 minutes, thereafter exposure to antigamma serum (dilution 1:1,000) for 4 minutes.

In adsorption tube

\[
5.3 \times 10^7 \text{ bacteria per ml} \\
4.2 \times 10^7 \text{ gamma per ml}
\]

After 5 minutes 0.1 ml of this mixture is diluted 1:10 in a tube containing 0.9 ml antigamma serum in dilution 1:1,000. This dilution effectively stops further adsorption. The serum reduces the free virus titer in 4 minutes by a factor 100. At 9 minutes a sample of this mixture is diluted further 1:100 with broth. This dilution effectively stops further action of the serum. The whole experiment is run in parallel with a control in which antiserum is omitted from the intermediate dilution tube.

**Burst size**

<table>
<thead>
<tr>
<th></th>
<th>In serum-treated tube</th>
<th>In control tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>142</td>
<td>120</td>
</tr>
</tbody>
</table>

Virus liberation in both cultures was complete at 30 minutes. This experiment, therefore, showed no effect of the antiserum on adsorbed gamma, or on gamma growing within the bacteria. It was thought that longer exposure to antiserum might show an effect on intracellular virus.
**Experiment 2.** The same procedure was followed as in the preceding experiment except that exposure to antiserum was continued up to 2 minutes before the beginning of lysis, that is, up to 19 minutes after infection.

**Burst size**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plaque Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>In serum-treated tube</td>
<td>170</td>
</tr>
<tr>
<td>In control tube</td>
<td>206</td>
</tr>
</tbody>
</table>

Virus liberation in both cultures was complete at 30 minutes.

The result shows that there is no effect of antigamma serum on intracellular virus if the serum treatment is continued up to 2 minutes before lysis.

It was thought that antiserum might show an effect if it were added sooner. In the following experiment antiserum was added 1.5 minutes after the virus. In order to insure that the bacteria would be infected as early as possible, virus was added greatly in excess of the bacteria, that is, multiple infection was used.

The following facts must be borne in mind in the interpretation of multiple infection experiments (cf. Delbrück and Luria, 1942). The plaque titer falls during the adsorption period because several virus particles combine with each bacterium, and each such bacterium forms only one plaque up to the moment of lysis. Suppose, for instance, that virus and bacteria are mixed in the ratio 25 to 1. Suppose, further, that during the adsorption period each bacterium picks up an average of 10 virus particles. A plaque count at the end of the adsorption period will then give 15 plaques caused by free virus particles for every plaque caused by an infected bacterium. During the adsorption period, therefore, the plaque titer will fall from 25 to 16, and will give essentially the free virus titer. If, however, after the adsorption period the free virus is eliminated by antiserum, the plaque titer will fall further, from 15 + 1 to 1, that is, by a factor 16, and the plaque count will give the number of infected bacteria. Since practically all the bacteria were infected, this plaque count should be comparable with a colony count of the bacteria from plates made just before the beginning of the experiment. These expectations are borne out by the following experiment:

**Experiment 3.** Multiple infection with gamma, adsorption for 1.5 minutes, exposure to antiserum (dilution 1:1,000) for 4.5 minutes.

In adsorption tube

- $6.5 \times 10^7$ bacteria per ml
- $180 \times 10^7$ gamma per ml

Input ratio, 28 virus particles per bacterium.

After 1.5 minutes a dilution of 1:10 was put into a tube containing antigamma serum in a dilution of 1:1,000. At 6 minutes a further dilution of 1:100 was made in broth. In the control serum was replaced by saline.

Results: In the culture which was exposed to antiserum a plaque assay at 12.5 minutes gave

- $13 \times 10^7$ plaques per ml

As pointed out above, this count should be compared with the bacterial count. It is twice as high as the bacterial count just before the beginning of the
experiment. Apparently the bacteria continued to divide for a few minutes after the virus was added.

A corresponding plaque count of the control culture gave

\[146 \times 10^7\] plaques per ml

Of these,

\[13 \times 10^7\] plaques per ml

are due to infected bacteria. The remainder,

\[133 \times 10^7\] plaques per ml

must be due to free virus particles. This is 74 per cent of the input. Therefore, 26 per cent were adsorbed, or 7.2 per bacterium.

In both the serum-treated and the control culture the plaque titer began to rise around 21 minutes, and had reached its maximum at 40 minutes.

**Burst size**

<table>
<thead>
<tr>
<th>In serum-treated tube</th>
<th>210</th>
</tr>
</thead>
<tbody>
<tr>
<td>In control tube</td>
<td>290</td>
</tr>
</tbody>
</table>

This is hardly a significant difference in view of the inaccuracy introduced by numerous rapid dilution steps. We conclude that there is no effect of antigamma serum on bound gamma when the antiserum is added 1.5 minutes after adsorption.

It seemed desirable to check these findings with other viruses, particularly since it is known that gamma is very powerful in interference experiments, in which it competes with other viruses. This might indicate that gamma penetrates into the host faster than the other viruses. Therefore, similar experiments were undertaken with virus alpha which, in interference, is completely suppressed by gamma.

*Experiment 4.* One to one infection with alpha, adsorption for 3 minutes, exposure to antialpha serum (dilution 1 to 10) for 4 minutes.

In adsorption tube

\[8.7 \times 10^7\] bacteria per ml

\[47 \times 10^7\] alpha per ml

After 3 minutes a dilution of 1:20 was put into a tube containing antialpha serum in a dilution of 1:10. This serum dilution reduces the free virus titer in 4 minutes by a factor of about 100. At 7 minutes a dilution of 1:100 was made in broth. The control experiment with serum was replaced by broth run in parallel. At 10 minutes

<table>
<thead>
<tr>
<th>In serum-treated culture</th>
<th>7 \times 10^7] plaques per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>In control culture</td>
<td>43 \times 10^7] plaques per ml</td>
</tr>
</tbody>
</table>

The difference between these values must be accounted for by free virus. It is 77 per cent of the input. The adsorbed fraction is, therefore, 23 per cent of
SPECIFIC ANTISERA AND GROWTH OF BACTERIAL VIRUSES

the input, or slightly more than one virus particle per bacterium. Virus liberation was complete in both cultures at 22 minutes.

Burst size

<table>
<thead>
<tr>
<th></th>
<th>Serum-treated tube</th>
<th>Control tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>In serum</td>
<td>233</td>
<td>236</td>
</tr>
</tbody>
</table>

We conclude that there is no effect of antialpha serum on bound alpha when the serum is added 3 minutes after the beginning of adsorption.

In all these experiments the serum treatment was adjusted to give an inactivation of free virus to about 1 per cent. It was thought that a higher concentration of a high titer serum might show some effect. Our highest titer serum was an antidelta serum and this virus was, therefore, used for the test.

Experiment 5. Multiple infection with delta, adsorption for 5.5 minutes, exposure to antiserum (dilution 1 to 10) for 2.5 minutes.

In adsorption tube

8.5 × 10⁷ bacteria per ml
38 × 10⁷ delta per ml

After 5.5 minutes a dilution of 1:20 was put into a tube containing antidelta serum in a dilution of 1:10. This serum concentration will reduce the free virus titer in 2.5 minutes by a factor of more than 1,000,000.

At 8 minutes a dilution of 1:100 was made into broth, for immediate plaque assay, and a further dilution of 1:200 was made for assay after virus liberation has occurred.

Results:

At 10 minutes

<table>
<thead>
<tr>
<th></th>
<th>Serum-treated culture</th>
<th>Control tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>In serum</td>
<td>8.7 × 10⁷ plaques per ml</td>
<td>14.4 × 10⁷ plaques per ml</td>
</tr>
</tbody>
</table>

The difference between these values is due to the presence of the free virus. It is 15 per cent of the input. The adsorbed fraction, therefore, is 85 per cent of the input, or 4 virus particles per bacterium.

Virus liberation in both cultures was complete in 20 minutes.

Burst size

<table>
<thead>
<tr>
<th></th>
<th>Serum-treated culture</th>
<th>Control culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>In serum</td>
<td>238</td>
<td>214</td>
</tr>
</tbody>
</table>

We conclude that exposure to antidelta serum under conditions which will reduce the titer of free delta by a factor greater than 1,000,000 has no effect on bound delta.

Within the limits of their accuracy these experiments reveal no effect of antivirus serum on adsorbed or intracellular virus. This result may be stated in more detail as follows: (1) No infected bacterium fails to lyse when antivirus serum is added. (2) The latent period between adsorption and lysis is not changed by antivirus serum. (3) The burst size is not changed by antivirus serum. The limits of accuracy are about 20 per cent for each of these statements.
GROWTH OF SERUM SURVIVORS

The inactivation of virus by antibody is not necessarily an all-or-none effect. In some cases the "serum survivors" show evidence of partial inactivation. The plaques which they form appear later and are smaller. The impression is gained that the partial inactivation consists largely in a delay of the onset of virus growth. It seemed worthwhile to explore this matter further by examining the growth of serum survivors in a one step growth experiment.

Experiment 6. Single infection with a gamma stock in which more than 99 per cent of the particles had been inactivated.

(a) Preparation of serum survivors

Antigamma serum (diluted 1:1,000) is mixed with gamma stock (diluted 1:4). The mixture is incubated for 17 minutes at 37 C.

<table>
<thead>
<tr>
<th>Input</th>
<th>340 $\times 10^7$ gamma particles per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors after 17 minutes</td>
<td>0.88 $\times 10^7$ gamma particles per ml</td>
</tr>
<tr>
<td>Survival</td>
<td>0.26 per cent</td>
</tr>
</tbody>
</table>

(b) Adsorption mixture

<table>
<thead>
<tr>
<th>Adsorbed after 5 minutes</th>
<th>85 per cent of active gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c) A control tube which is run in parallel with untreated gamma particles contains the same concentration of bacteria and a number of gamma particles corresponding to the active fraction above.</td>
<td></td>
</tr>
<tr>
<td>Adsorbed after 5 minutes</td>
<td>93 per cent of active gamma</td>
</tr>
</tbody>
</table>

Equal growth was found in tubes (b) and (c). In both, liberation started at 21 minutes, and the plaque titer increased proportionately in both tubes. This experiment, therefore, failed to reveal any partial inactivation of the serum survivors. It is, however, unlikely that this result has general validity. It may well be that with other viruses or with other conditions of serum inactivation evidence for partial inactivation will be found. The experiment merely shows that under the given conditions the survivors are as effective as normal gamma particles, although they undoubtedly have combined with many antibody molecules.6

DOES INACTIVE VIRUS INTERFERE WITH THE GROWTH OF ACTIVE VIRUS?

The term "inactivation of virus," as here defined, means that the particles fail to form plaques. The experiments thus far do not tell us whether or not the particles are still able to attach themselves to their hosts. It is conceivable that they are adsorbed, and if adsorption takes place interference with the growth of active virus of the same or of a different strain might occur. A similar phenomenon has been observed to take place with virus which is inactivated by ultraviolet light (Luria and Delbrück, 1942).

The following two experiments show that the presence of serum-inactivated virus gamma does not interfere with the growth of either active gamma or active alpha:

Experiment 7. Single infection with active gamma in the presence of an excess of inactive gamma.
(a) Preparation of inactive virus

Antigamma serum (dilution 1:2,000) is mixed with gamma stock (dilution 1:4). The mixture is incubated for 20 minutes at 37 C.

<table>
<thead>
<tr>
<th>Input</th>
<th>$440 \times 10^7$ gamma particles per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors after 17 minutes</td>
<td>$5.8 \times 10^7$ gamma particles per ml</td>
</tr>
<tr>
<td>Survival</td>
<td>1.3 per cent</td>
</tr>
</tbody>
</table>

(b) Adsorption mixture

<table>
<thead>
<tr>
<th>Input ratios</th>
<th>12 total gamma per bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorbed after 5 minutes</td>
<td>0.15 active gamma per bacterium</td>
</tr>
<tr>
<td>Adsorption after 5 minutes</td>
<td>78 per cent of active gamma</td>
</tr>
</tbody>
</table>

Results: Liberation of gamma began at 21 minutes and was complete at 35 minutes.

Burst size 90 gamma particles per bacterium

This burst size is not significantly lower than the burst size for untreated gamma. It may be concluded that inactive gamma, though twelvefold in excess of the bacteria, did not interfere with the growth of active gamma. The experiment does not indicate whether or not the inactive gamma was adsorbed. It merely shows that if the inactive virus was adsorbed it did not interfere with the growth of the active virus.

Experiment 8. Single infection with alpha in the presence of an excess of inactive gamma.

Preparation of inactive gamma as in the preceding experiment. Survival 0.5 per cent.

<table>
<thead>
<tr>
<th>Adsorption mixture</th>
<th>$7.3 \times 10^7$ bacteria per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$7.0 \times 10^7$ alpha per ml</td>
</tr>
<tr>
<td></td>
<td>$20 \times 10^7$ total gamma per ml</td>
</tr>
<tr>
<td></td>
<td>$0.1 \times 10^7$ active gamma per ml</td>
</tr>
<tr>
<td>Input ratios</td>
<td>1 alpha per bacterium</td>
</tr>
<tr>
<td></td>
<td>2.7 total gamma per bacterium</td>
</tr>
<tr>
<td></td>
<td>0.01 active gamma per bacterium</td>
</tr>
</tbody>
</table>

Adsorption after 5 minutes 66 per cent of alpha

Results: A plaque count of alpha at 8 minutes (before the beginning of lysis) gave $6.4 \times 10^7$ plaques per ml, approximately the same as the input. This shows that all bacteria infected with alpha liberated alpha. Liberation of alpha was complete at 20 minutes. Burst size was 135. It may be concluded that serum-inactivated gamma does not interfere with the growth of alpha.

**EFFECTS OF ANTIBACTERIAL SERUM ON VIRUS GROWTH**

The antivirus sera used in the preceding experiments had been prepared by injecting filtered lysates into rabbits. The lysates contain, besides virus, much bacterial debris. The antisera, therefore, contain a certain number of antibacterial antibodies. The homologous bacteria were weakly agglutinated by the antivirus sera to a titer of 320. The agglutinins were absorbed from the sera.
with suspensions of homologous bacteria. The antivirus titers were not changed by this procedure.

In some of the experiments on interference, described in a subsequent paper, in which antivirus sera were used to eliminate free virus, irregularities were found which could be traced to the antibacterial components of the sera. It seemed likely that the agglutinins caused these effects by combining at specific sites on the bacteria, thus blocking them from the virus. The series of experiments recorded below was designed to analyze these effects using a high titer antibacterial serum.

**Experiment 9.** Adsorption and growth of virus delta on bacteria treated with antibacterial serum.

**TABLE 1**

<table>
<thead>
<tr>
<th>EXP.</th>
<th>SERUM DILUTION</th>
<th>% ADSORPTION IN 3 MINUTES</th>
<th>VIRUS GROWTH IN 50 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>20</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2a</td>
<td>40</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>2b</td>
<td>80</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>3a</td>
<td>100</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>3b</td>
<td>200</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>4a</td>
<td>190</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>4b</td>
<td>600</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>1a</td>
<td>control (no serum)</td>
<td>60</td>
<td>200</td>
</tr>
</tbody>
</table>

Each of a series of serum dilutions was tested in a separate one step growth experiment. The experiments were run in pairs (a and b) from the same growing culture. The serum was added to the bacterial culture 8 minutes before adding the virus.

Table 1 lists the percentage of adsorption and the delta titer at 50 minutes. The table shows clearly the expected effect. Up to an 80-fold dilution of serum, practically no virus adsorption and very little growth of virus occurred. At higher dilutions of the serum virus growth and adsorption return to the control values. The transition is remarkably sharp. This may be seen from the pair in which the serum dilutions of 100 and 200 were run in parallel. At the dilution of 200, growth and adsorption are normal; whereas at the dilution of 100, growth is about seven times less and adsorption is barely measurable.

Similar effects were observed with alpha and gamma. With both of these viruses adsorption and growth were largely but not completely suppressed by antiserum at a dilution of 100.

This technique affords a method of following the progress of the reaction between the bacterial cell and the agglutinins in the antiserum. This reaction must be very rapid, because in an experiment in which antiserum at a dilution...
of 40 was added to the bacteria only 1 minute prior to the addition of virus, complete suppression of virus adsorption and growth was found to take place.

TITRATION OF ANTIBACTERIAL SERUM BY MEANS OF ITS INHIBITING INFLUENCE UPON LYSIS

The inhibition of virus adsorption may be utilized in the titration of the antiserum by determining the highest dilution of the antiserum which will delay the appearance of mass lysis in a tube inoculated with a growing culture of bacteria and an excess of virus.

Experiment 10. Titration of antibacterial serum.

TABLE 2
Titration of antibacterial serum by means of its inhibiting influence upon lysis of the bacteria by virus

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>DILUTION OF SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Alpha</td>
<td>+</td>
</tr>
<tr>
<td>Delta</td>
<td>+</td>
</tr>
</tbody>
</table>

TABLE 3
Retarding influence of higher dilutions of serum on the growth of adsorbed virus delta

<table>
<thead>
<tr>
<th>TIME IN MINUTES</th>
<th>SERUM DILUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
</tr>
</tbody>
</table>

Procedure: A series of tubes containing decreasing amounts of antibacterial serum is set up. Equal amounts of a broth suspension of live growing bacteria is added to each tube, so as to give a clearly visible turbidity. This series is incubated for 5 minutes. An equal amount of virus is then added to each tube, the number of virus particles added being about fivefold in excess of the number of bacteria. Two tubes without antiserum, one with bacteria only, the other with bacteria and virus, are set up as controls. The purpose of these tubes is to indicate the onset of mass lysis. As soon as the control tube with bacteria and virus shows the onset of lysis, the entire series of tubes is compared with it. The first members of the series will be more turbid than this control tube, because lysis has been inhibited by the antiserum. The highest dilution of antiserum which causes such a delay of lysis may be referred to as the "lysis inhibition titer" of the antiserum.

Table 2 shows the results of an experiment of this type in which the same serum was titered in parallel with viruses alpha and delta.
It will be seen that the titrations obtained with two different viruses agree fairly closely. The readings were taken 35 minutes after the addition of virus. If readings are taken at a later time the titers may be somewhat lower. Apparently at the limiting serum dilutions the serum does not prevent or delay adsorption of virus, but it delays the action of adsorbed virus. Therefore, given sufficient time these tubes will reach complete lysis as does the control tube. At later readings these tubes will then be recorded as negatives.

This delaying action of serum on adsorbed virus was also in evidence in the one step growth experiments listed in table 1. The virus growth listed in this table was that observed 50 minutes after infection. Plaque counts were also made from samples taken 20 minutes and 30 minutes after infection. In the controls and in the experiments with very high dilutions of serum the plaque counts after 30 minutes were as high as those after 50 minutes. This means that lysis was complete in 30 minutes. In the experiments with serum dilutions of 100 and 200, however, the plaque counts at 30 minutes were much lower than those at 50 minutes (table 3).

**DISCUSSION**

Within the limits of their accuracy our experiments with antivirus sera reveal a remarkably simple situation. Adsorbed and intracellular virus appears to be completely insusceptible to the action of strong specific antivirus serum. Although this is by no means an unexpected result, the extraordinary difference in sensitivity between free and bound virus could hardly have been predicted on the basis of previous observation or of current theories. In a subsequent paper experiments will be presented which confirm and amplify these findings by more sensitive tests, and which indicate a very slight and transient residual sensitivity of bound virus to antivirus serum, too slight to be evident in the tests here reported.

Our experiments fail to reveal evidence of partial inactivation in the serum survivors. The conditions of inactivation in these experiments were such that a large part of the surface of the survivors must have been coated with antibodies. The prompt growth of these partially coated virus particles probably means that the adsorbed antibodies are quickly removed when the virus enters the bacterium. Our experiments do not indicate the mechanism of this removal. It may be enzymatic in nature, resembling the reactivation by papain (Kalmanson and Bronfenbrenner, 1943), or it may be similar to the dissociation of the antigen-antibody complex by electrolyte, as in the experiments of Heidelberger and Kabat (1938) on the dissociation of antibody from pneumococcus-specific precipitates. Whatever the mechanism may be, it is probably a factor which contributes to the resistance of bound (adsorbed or intracellular) virus to the action of antiserum.

The fact that serum-inactivated virus does not interfere with the growth of active virus of the same strain or of a different strain probably means that the inactive virus (under our experimental conditions) is very little, if at all, adsorbed by the host cell. If it were adsorbed one would expect it to grow and interfere, since the serum survivors grow without inhibition.
SPECIFIC ANTISERA AND GROWTH OF BACTERIAL VIRUSES

The experiments with antibacterial serum also reveal a feature which predominates at high serum concentrations, namely, the blockade of adsorption caused by the coating of the bacterial surface with bacterial antibodies. The speed of this reaction (complete coating in 1 minute) is not surprising, since Mayer and Heidelberger (1942) found even faster rates for the reaction of antibodies with specific polysaccharides of pneumococcus.

It is likely that the blocking effect of antibacterial serum on the adsorption of virus is highly specific and that it could be applied to a detailed study of bacterial antigenic structure. It is well known that variant bacterial strains which are resistant to a certain virus do not adsorb the virus. It has been supposed, and in some instances it has been proved, that this failure to adsorb the virus is connected with a change in the antigenic structure of the host. It may be assumed that the surface elements of the sensitive strain which are responsible for the adsorption of the virus (the “receptor spots”) are so altered in structure in the resistant bacterial strain that they fail to accept the virus. One would expect a corresponding change in the antibodies elicited by the sensitive and the resistant bacterial strains, respectively. Absorption of antiserum to the sensitive strain by resistant bacteria will remove all antibodies except the differential ones. Antiserum absorbed in this manner should, therefore, be highly specific for the blocking of those viruses which are adsorbed by the differential antigens.

SUMMARY

Exposure of virus-infected bacteria to strong antivirus serum fails to affect the course of virus growth.

The survivors of a suspension of gamma virus, more than 99 per cent of which is inactivated, are adsorbed as rapidly as are untreated gamma particles. There is also no detectable difference between the growth rates of these serum survivors and of untreated virus particles.

Gamma virus inactivated by antiserum fails to interfere with the growth of untreated gamma virus or of alpha virus.

Adsorption of virus is strongly inhibited if the bacteria are pretreated for a few minutes with antibacterial serum. This effect can be utilized for rapid titrations of antibacterial serum.

Bacteria treated with high dilutions of antibacterial serum will adsorb virus, but there is a slight delay in the liberation of virus from these bacteria.

REFERENCES


