THE PRODUCTION OF STREPTOCOCCUS HEMOLYTICUS
BACTEREMIA IN NON-SPECIFICALLY SENSITIZED
ANIMALS

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During the course of studies concerning the relation of the hyper-
sensitive state to localization of bacteria, Weisberger (1) observed that
there was a prolongation of bacteremia in rabbits previously sensitized
to normal horse serum. Rabbits hypersensitive to horse serum were
inoculated simultaneously with a sublethal quantity of Streptococcus
viridans following which blood cultures were positive for many hours,
in some instances as long as 72 hours. On the other hand, in non-sen-
sitized animals that received a similar inoculation of horse serum and
bacteria, the organisms disappeared in 1½ to 2 hours after inoculation.

Due to the frequency of a sensitized condition of individuals to horse
serum and the fact that antisera prepared in the horse are employed
as therapeutic agents in certain infectious diseases, is it not possible
that a similar phenomenon may be the basis of the change observed
frequently in the clinical course of these diseases following shortly
after the injection of antiserum?

As an approach to study of the subject, experiments were designed
to observe the behavior of non-specific and specific sensitized animals
with respect to bacteremia following simultaneous inoculation of anti-
gen and a scarlet fever strain of hemolytic streptococci. It is the pur-
pose of this communication to present the results of experiments in
which antisera prepared in the horse were employed as antigens to in-
ject simultaneously with the bacteria into rabbits sensitized to normal
horse serum.

EXPERIMENTAL

A 20 to 22 hour culture of a hemolytic streptococcus recovered from a case of
scarlet fever (Strain 273) was used throughout these experiments. Studies of the
rate of growth of this streptococcus in broth showed the maximum period of active
growth to occur between the 8th and 10th hours. Depending on the quantity of
culture desired, 1 or 2 cc. of the broth culture was centrifuged, the supernatant
liquid decanted and the bacterial sediment resuspended in 10 cc. of sterile saline.
Exactly 1 cc. of the suspension was injected into the ear veins of normal and sensi-
tized animals from the same syringe. In order to eliminate factors such as change
in virulence\(^1\) of the organism, difference in the quantity of bacteria and variations
in volume injected, the control and the sensitized animals were injected as nearly
simultaneously as possible. The quantity of suspension injected was kept con-
stant at 1 cc. for the entire series. In order to guard against change in bacterial
growth upon standing, the whole procedure was so timed that the interval between
the removal of the broth culture from the incubator and the injection of the suspen-
sion was never greater than 20 minutes.

All the rabbits were obtained from the same source, and in each pair of animals
run simultaneously, the weight variation was never greater than 2/5 kilo. Those
animals selected for sensitization were given three intraperitoneal injections of
normal horse serum at 4 day intervals. After a period of 18 to 21 days the animals
were skin tested for sensitivity by intracutaneous injection of normal horse serum
in dilutions of 1:5 and 1:10. The skin reactions were controlled by intracutaneous
injection of normal saline. Those animals giving strongly positive skin reactions
to both dilutions as evidenced by local erythema and edema were taken for experi-
mental use. Animals giving equivocal or negative skin reactions were discarded
with one exception. This animal was used as the control as shown in Table 1.
The kinds of sera used in this study and their source are as follows: (1) normal
horse serum, (2) scarlet fever antitoxin and (3) diphtheria antitoxin (Eli Lilly
and Company—without preservative).

From 7 to 9 days after operation, the animals were sacrificed with ether and
necropsies performed. Cultures were made from the liver, the heart's blood and
the spleen. In certain of the animals, histological sections of the tissues were
made and routine hematoxylin-eosin and Gram stains were prepared.

A typical protocol of an experiment is given.

August 31, 11.30 a.m. Rabbit 1, weighing 1600 gm.; ear vein shaved. Rabbit
2, weighing 2000 gm.; shaved, prepared and the jugular vein exposed under ether
anesthesia. 12.55 p.m. Operation complete. 1.00 p.m. Culture removed from
incubator. 2 cc. transferred to sterile centrifuge tube and centrifuged 10 minutes.
1 cc. transferred from same pipette to 9 cc. of sterile saline in first tube of dilution
series. Dilutions run out to \(10^{-2}\). 1.00 p.m. Plate poured from each dilution.

\(^1\) Virulence.—1 cc. of a 24 hour broth culture killed an albino mouse in a dilution
of \(10^{-1}\) (500 million per cc.) within 17 hours. With higher dilutions all the mice
survived and when autopsied on the 7th day, gave uniformly negative cultures.
Virulence as tested by this method was the same at the end as at the beginning of
the experimental period.
Dose to be calculated from these counts. 1.12 p.m. Supernatant liquid decanted and organisms resuspended in 10 cc. of saline. 1.15 p.m. Rabbit 1 injected in ear vein with 1 cc. of saline suspension. 1.17 p.m. Rabbit 2 injected in ear vein with 1 cc. of saline suspension (from same syringe) and a few seconds later injected in same ear vein with 1 cc. of 1:10 normal horse serum. No symptoms of shock noted. 1.30 p.m. Blood drawn from jugular of Rabbit 2. A few drops transferred to broth. 1 cc. put in plate and plate poured.

Following this, blood was drawn from the jugular vein in the same manner at 30 minutes, then every hour for the first 3 hours and finally at 6, 9, 12 and 24 hours. The incisions were closed with a few sutures and the animals returned to their cages after the 3 hour period and after every subsequent bleeding. After the 24 hour period, blood cultures were taken by means of cardiac puncture.

TABLE I

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Amount of horse serum</th>
<th>Quantity of organisms inoculated per cc.</th>
<th>Result of blood cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>millions</td>
<td></td>
</tr>
<tr>
<td>1 (n)</td>
<td></td>
<td>1.6</td>
<td>22</td>
</tr>
<tr>
<td>2 (s)</td>
<td>1 cc. of 1:10</td>
<td>1.6</td>
<td>34</td>
</tr>
<tr>
<td>3 (n)</td>
<td></td>
<td>1.6</td>
<td>20</td>
</tr>
<tr>
<td>4 (s)</td>
<td>1 cc. of 1:10</td>
<td>1.6</td>
<td>26</td>
</tr>
<tr>
<td>5 (n)</td>
<td></td>
<td>6.0</td>
<td>10</td>
</tr>
<tr>
<td>6 (s)</td>
<td>1 cc. of 1:10</td>
<td>6.0</td>
<td>75</td>
</tr>
<tr>
<td>7 (n)</td>
<td></td>
<td>0.1</td>
<td>4</td>
</tr>
<tr>
<td>8 (s)</td>
<td>1 cc. of 1:10</td>
<td>0.1</td>
<td>15</td>
</tr>
</tbody>
</table>

n, normal; s, sensitized.

RESULTS

The results observed in four non-sensitized and four sensitized rabbits in which bacteremias were studied are presented in Table I.

Blood cultures of the non-specifically sensitized animals remained positive for a longer period than those of the non-sensitized group. The periods of prolongation varied from 4 to 15 hours, but seemed to bear no relation to the initial quantity of organisms injected. Rabbit 3 had received sensitizing injections of horse serum but failed to give a positive skin reaction. Although the duration of the positive blood cultures in this animal was strikingly longer than in other controls,
the sensitized rabbit that received the horse serum and organisms showed positive cultures for a significantly longer time. Whether those animals that fail to show a skin sensitivity react differently from normal animals remains for further investigation. However, these experiments indicate that in rabbits that are non-specifically sensitized there is a tendency for prolongation of the duration of *Streptococcus hemolyticus* in the circulation when horse serum is inoculated simultaneously with the suspension of bacteria.

In order to study the effects of normal horse serum in altering the response of a non-sensitized rabbit to the inoculation of *Streptococcus hemolyticus*, two rabbits were inoculated simultaneously with normal serum and a known suspension of bacteria. The results of this experiment are shown in Table II.

Simultaneous injections of normal horse serum and bacteria do not prolong the bacteremia in non-sensitized animals. When the quantity of the initial inoculation approaches the maximum limit or the lethal dose, the variations in duration of positive blood cultures is more evident. This fact emphasizes the necessity for use of quantitative methods in such a study.

The next group of animals includes studies in which scarlet fever and diphtheria antitoxins in horse serum are employed instead of normal horse serum. Both the non-sensitized and sensitized animals received similar injections of serum and organisms, as shown in Table III.

Table IV shows the rate of disappearance of the organism from the blood stream in one of these experiments.
### TABLE III

<table>
<thead>
<tr>
<th>Series III</th>
<th>Amount of streptococcus antitoxin</th>
<th>Quantity of organisms inoculated per cc.</th>
<th>Result of blood cultures</th>
<th>Maximum No. of colonies per cc.</th>
<th>Duration of positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (n)</td>
<td>1 cc. of 1:10</td>
<td>1.0</td>
<td></td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>10 (s)</td>
<td>1 cc. of 1:10</td>
<td>1.0</td>
<td></td>
<td>94</td>
<td>10</td>
</tr>
<tr>
<td>11 (n)</td>
<td>1 cc. of 1:1</td>
<td>3.6</td>
<td></td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>12 (s)</td>
<td>1 cc. of 1:1</td>
<td>3.6</td>
<td></td>
<td>620</td>
<td>12</td>
</tr>
<tr>
<td>13 (n)</td>
<td>1 cc. of 1:1</td>
<td>7.0</td>
<td></td>
<td>34</td>
<td>48</td>
</tr>
<tr>
<td>14 (s)</td>
<td>1 cc. of 1:1</td>
<td>7.0</td>
<td></td>
<td>132</td>
<td>48</td>
</tr>
</tbody>
</table>

### TABLE IV

<table>
<thead>
<tr>
<th>Time after inoculation</th>
<th>Colonies per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series III</td>
</tr>
<tr>
<td></td>
<td>No. 9</td>
</tr>
<tr>
<td>min.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>hrs.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

### TABLE V

<table>
<thead>
<tr>
<th>Series IV</th>
<th>Amount of diphtheria antitoxin</th>
<th>Quantity of organisms inoculated per cc.</th>
<th>Result of blood cultures</th>
<th>Maximum No. of colonies per cc.</th>
<th>Duration of positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (s)</td>
<td>1 cc. of 1:1</td>
<td>0.4</td>
<td></td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>16 (s)</td>
<td>1 cc. of 1:1</td>
<td>0.4</td>
<td></td>
<td>263</td>
<td>24</td>
</tr>
</tbody>
</table>
Although the difference in the prolongation of the bacteremia in the sensitized animals from the non-sensitized animals is only 0 to 4 hours in these experiments, there is, nevertheless, a definite increase in the actual number of organisms present in the blood cultures of the sensitized animals.

When diphtheria antitoxin horse serum is employed, similar results are obtained, as shown in Table V.

No evidences of gross anatomical change appeared in any of the animals. The only significant microscopic changes in the viscera were found in the kidneys where proliferation of fibroblasts, infiltration of small round cells, a few capsular adhesions and areas of hemorrhage were more marked in the sensitized animals. Gram stains of the tissues were uniformly negative for bacteria, which was consistent with the negative cultural findings obtained at necropsy.

DISCUSSION

The results of our experiments present evidence in support of Weisberger's conclusions; namely, that a prolonged bacteremia occurs in the hypersensitive animal, the minimal duration being 9 hours as compared with 3 hours in the non-sensitized control animal, although the actual maximum and minimum colony counts in these animals exhibit no significant differences. Also, these results agree with those of Clawson (2) who has shown that streptococci are removed less rapidly from the blood stream of specifically sensitized rabbits than from the blood stream of normal rabbits within an interval of 15 minutes. In reviewing the literature further concerning studies on experimental bacteremia in animals, it is surprising that few investigators apparently have had their interests engaged by the study of bacteremia in relationship to sensitization. In fact, the only other report bearing on this subject is that of Boone, Chase and Brink (3) who found that large numbers of \textit{B. prodigiosus} are absorbed from the intestinal tract of dogs during an acute anaphylactic shock.

In our experiments we have extended the observations on experimental bacteremia to the study of the influence of antisera employed as antigens instead of plain horse serum. As shown by the data presented the actual increase in the number of bacteria for the periods studied is significantly greater in the sensitized animal, yet the duration
of the bacteremia is approximately the same as is observed in the normal animal, in contrast to the prolonged bacteremia of the sensitized animal shocked by means of normal horse serum. Since it is generally believed that bacteria are in large part removed from the blood by the cells of the reticulo-endothelial system in the liver and spleen of animals, it is suggested that the sensitization process alters this attribute of these cells so that they become inefficient in removing the bacteria rapidly.

However, in the experiments we have described it is only during the interval of the first few hours that greater bacteremia is observed in the animals shocked with antisera. Therefore, if it be assumed that the sensitization process affects the cells in the manner just stated, it is necessary to assume further that there is a differential action between normal horse serum and antisera. As a matter of fact, the differences in the reaction of animals sensitized to horse serum when shocked with antisera contrasted with plain horse serum have never been studied in detail. Further analyses of this phenomenon are in progress at the present time.

CONCLUSIONS

Rabbits sensitized to horse serum developed a bacteremia of 9 to 12 hours' duration when they were inoculated simultaneously with normal horse serum and a strain of Streptococcus hemolyticus, while the bacteria could only be isolated from the blood stream of non-sensitized animals within the first 3 hours after inoculation. On the other hand, when antisera are employed as the antigen for shocking the sensitized rabbits, there is a significant increase in the number of bacteria in the blood stream in contrast to the control animals, but there is no evidence of a prolongation of the bacteremia.

BIBLIOGRAPHY