The problem of the effective treatment of anthrax infection in man is still unsettled. The failure to attain consistent satisfactory results with the various methods at hand has led to the search for other remedies. The use of anti-anthrax serum has received support from one group of observers while others have been skeptical as to its efficiency. The value of serum was questioned by Penna, Cuenca and Kraus 1 and others who believed that it was inferior in its therapeutic efficiency to that of normal bovine serum. Destéfano and Vaccarezza 2 have shown that the nonspecific shock produced by the injection of peptone was of more value than either immune serum or beef serum in the treatment of anthrax.

Attention has also been devoted to the chemotherapy of anthrax. Many drugs have been advocated as specific in the treatment of the disease but none have been found to be of undisputed value. One of the most recent drugs used in the treatment of anthrax is salvarsan which was first suggested and used by Becker 3 in 1911. Since then many reports both experimental and clinical have appeared in which satisfactory results have been obtained. However, Becker himself 4 found that salvarsan was not always effective clinically and Cambessedies and Reilly 5 claimed that it was of no value in combating experimental anthrax infection in guinea-pigs. It is difficult to evaluate the effectiveness of specific immune therapy or of chemotherapy of anthrax since the statistics in regard to the mortality are so variable and divergent, varying from 4 to 37%. 6 Spontaneous recovery also occurs.

Received for publication, Sept. 26, 1929.

5. La Presse Méd., 1925, 33, p. 969.
A case of cutaneous anthrax reported by Canright in this hospital was apparently successfully treated with neosalvarsan. Since his report was published two other cases have been observed. One, an advanced case with a pustule on the neck, died in spite of intensive treatment with both immune serum and neosalvarsan. The other, an early case with a pustule on the eyelid, recovered after receiving .6, .9, and .9 gm. of neosalvarsan on three successive days. An experimental study was then made to determine the comparative value of anti-anthrax serum and of neosalvarsan in treating anthrax in mice, guinea-pigs and rabbits.

Methods.—The strain of anthrax bacilli used in this study was obtained from the pustule of a fatal case of anthrax. Mice died within 2 days after the subcutaneous inoculation of .000001 cc. of a 24-hour agar slant growth suspended in 5 cc. physiologic salt solution. Guinea-pigs succumbed within 3 days after inoculation with .000005 cc. Two kinds of commercial anti-anthrax serums made by well known companies and the neosalvarsan of Billon were used in the tests.

As a rule the virus was inoculated subcutaneously so that .2 cc. of physiologic salt solution contained the desired amount of bacilli for mice, and 0.5 cc. for guinea-pigs and rabbits. Anti-anthrax serum and neosalvarsan were injected intravenously into the tail veins of mice, heel veins of guinea-pigs and the ear veins of rabbits. The neosalvarsan was dissolved in sterile physiologic salt solution immediately before use.

Experiments with Mice.—It is well known that anti-anthrax serum is of no value in protecting mice from anthrax infection since these animals are so highly susceptible. In spite of this, however, comparative experiments were made to determine if either anti-anthrax serum or neosalvarsan had any therapeutic effect whatever in prolonging life.

Eighty-five white mice, averaging 15 grams in weight, were infected with varying amounts of bacilli (from 1:500000 to 1:1000000 cc.). Serum was administered intravenously in varying amounts corresponding to 1 cc. to 13 cc. per kilo of weight. In several instances serum was given immediately after inoculation with bacilli but usually from ½ hour to 3 days before infection. Without exception all animals died within 24 to 36 hours from anthrax septicemia.

In another group of 25 mice the therapeutic effect of neosalvarsan was tested. In spite of the findings of Bettmann and Laubenheimer who used salvarsan in their tests, neosalvarsan was found not to be

toxic for mice. Preliminary titrations of the toxicity indicated that a
dose corresponding to .15 gm. per kilo given intravenously had no ill
effects.

Mice were then given .000005 cc. of anthrax bacilli subcutaneously
and were immediately injected with varying doses of neosalvarsan
(0.1 gm. to 0.025 gm. per kilo) intravenously. Within 36 hours all
of the animals succumbed to anthrax septicemia.

It is evident from these experiments that the intravenous injections
of large doses of anti-anthrax serum or of neosalvarsan were of no
therapeutic value in mice infected subcutaneously with anthrax bacilli.

**The Comparative Effects of Neosalvarsan and Anti-Anthrax Serum in the Treatment
of Experimental Anthrax Infections in Guinea-Pigs**

<table>
<thead>
<tr>
<th>Number of Guinea-Pigs</th>
<th>Dose of Virus Subcutaneously</th>
<th>Therapy (Intravenous)</th>
<th>Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1:5000</td>
<td>Neosalvarsan 2.5 mg. per kilo</td>
<td>Died in 3 days</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1:500</td>
<td>Neosalvarsan 5 mg.</td>
<td>2 died in 3 days</td>
<td>9 died, 12 survived</td>
</tr>
<tr>
<td>11</td>
<td>1:500</td>
<td>Neosalvarsan 10 mg.</td>
<td>5 died in 3 days</td>
<td>9 died, 12 survived</td>
</tr>
<tr>
<td>5</td>
<td>1:500</td>
<td>Neosalvarsan 20 mg.</td>
<td>6 survived</td>
<td>9 died, 12 survived</td>
</tr>
<tr>
<td>6</td>
<td>1:5000</td>
<td>1 cc. antiserum per kilo</td>
<td>All died within 24 hours</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1:5000</td>
<td>4 cc. antiserum per kilo</td>
<td>All died within 36 hours</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1:10,000</td>
<td>5 cc. antiserum per kilo</td>
<td>All died within 60 hours</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1:20,000</td>
<td>6 cc. antiserum per kilo</td>
<td>All died within 72 hours</td>
<td>26 died</td>
</tr>
<tr>
<td>5</td>
<td>1:50,000</td>
<td>10 cc. antiserum per kilo</td>
<td>All died within 72 hours</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1:1000 to 1:10,000</td>
<td>Untreated controls</td>
<td>All died within 70 hours</td>
<td>8 died</td>
</tr>
</tbody>
</table>

**Experiments with Guinea-Pigs.**—Similar unsuccessful attempts were
made to prevent death from anthrax in guinea-pigs by the therapeutic
administration of immune serum. Essentially the same technic was
employed as in the previous experiments. The quantities of bacilli and
anti-anthrax serum which were used are indicated in the accompanying
table. All 26 guinea-pigs treated with serum died within 72 hours
(table).

In accordance with the observations of other investigators,7, 8, 9
much more encouraging results were obtained when neosalvarsan was
given. In our experiment 24 guinea-pigs were used. The quantity of
bacilli inoculated was greater than in the preceding experiment as shown
in the table. The drug was injected intravenously immediately after
inoculation of the bacilli. The results are illustrated in the table.

It appears that 2.5 mg. of neosalvarsan per kilo of body weight is insufficient but that doses larger than this were of definite value in preventing a fatal outcome. Of the 21 animals receiving the larger doses 12 survived and 9 died in 3 or 4 days. All of the untreated controls died.

It is clear therefore that in guinea-pigs, neosalvarsan is of value in the treatment of anthrax infection since many of the animals survived after one injection of the drug although comparatively large quantities of virulent bacilli were given.

Experiments with Rabbits.—Since rabbits are even more resistant than guinea-pigs to artificial anthrax infection, it was expected that the therapeutic administration of neosalvarsan would be still more strikingly beneficial and that immune serum would also be of some value. Schuster 10 has already reported the successful treatment of rabbits with salvarsan. Twelve rabbits were given doses of anthrax bacilli similar to those given to guinea-pigs and immediately injected with immune serum. From 1 to 10 cc. of serum per kilo was given. All of the animals died within 5 days from anthrax septicemia.

The effect of neosalvarsan was then tried. Sixteen rabbits received the same amount of bacilli as in the preceding experiment and were then injected with amounts of neosalvarsan ranging from 2.5 mg. to 20 mg. per kilo. Within 8 days all of the animals succumbed to the infection.

Neither immune serum nor neosalvarsan then has any effect on the course of anthrax infection in rabbits, although death was delayed in a few of the animals receiving the drug.

Bactericidal Effect of Neosalvarsan in Vitro.—Several investigators 8 expressed the belief that salvarsan had a direct specific bactericidal action on anthrax bacilli. Ermilow and Golotina 11 found that anthrax bacilli were killed by salvarsan in dilutions of 1:5000 which they claimed corresponded to the concentration which they attained in human cases treated with their technic. Their experiments in vitro, however, were made in the absence of serum which reduces the value of their tests.

Experiments were then made to determine the bactericidal action in vitro of neosalvarsan dissolved in physiologic salt solution alone and in the presence of normal guinea-pig serum and normal rabbit serum. Three sets of tubes were arranged. The first set contained

neosalvarsan in ascending dilutions from 1:100 to 1:10,000 in physiologic salt solution. The second set contained the same dilutions of the drug in physiologic salt solution and 10% of normal rabbit serum and the third set contained the drug dissolved in physiologic salt solution and 10% of normal guinea-pig serum. After inoculating all tubes with a light suspension of anthrax bacilli derived from a 24-hour agar slant culture, they were placed in the incubator at 37 C. At the end of one hour and again after 2 hours transfers were made from all tubes into separate agar slants and incubated for 24 hours.

Results.—According to the growth obtained on the agar slants, viable anthrax bacilli were present in all tubes even after two hours incubation with a 1:100 dilution of neosalvarsan in physiologic salt solution. The growth on the agar slants was heavier in the tubes seeded from the suspensions incubated with higher dilutions of neosalvarsan and from the tubes containing serum. Normal rabbit and guinea-pig serum therefore exerted slight protective action on the bacilli against the effects of the drug. Similar results were obtained on repetition of the experiment.

Although anthrax bacilli were subjected to much stronger concentrations of neosalvarsan than those reported in the experiments by Ermilow and Golotina no striking bactericidal effects were noted.

DISCUSSION

Several investigators have drawn attention to the use of salvarsan preparations in the treatment of the anthrax. Numerous reports indicate that the drug is of value in the treatment of experimental and natural anthrax infections, although the number of patients treated in this manner is still small. In this hospital 3 cases of cutaneous anthrax received neosalvarsan and 2 recovered. In order to obtain further information in regard to the comparative value of anti-anthrax serum and of neosalvarsan, experiments were made on the treatment of anthrax infections in laboratory animals.

It was found that the specific immune serums which were used had no therapeutic value in experimental anthrax infections in mice, guinea-pigs and rabbits. But it cannot be assumed from this that serum is of little or no value in the treatment of anthrax in other species, since it has been found to be of undoubted value in other domestic animals. Moreover numerous statistics exist indicating its usefulness in treating human anthrax. The frequent failure of this method of treatment, however, has led to a search for other remedies which are more effective.
According to the results of these experiments neosalvarsan was only of value in the treatment of experimental anthrax infections in guinea-pigs and without any effect in mice and rabbits. It was disappointing to find that the drug failed to influence the infection in rabbits since these animals are naturally more resistant to anthrax bacilli than guinea-pigs. It is obvious then that it is difficult to predict the value of any measures in the treatment of human anthrax infections from the results of experiments on other animals. Each species apparently behaves in a different manner to therapeutic efforts. Therefore to evaluate the treatment of anthrax infections in human beings with salvarsan it will be necessary to gather the statistics from many more cases treated with the drug.

CONCLUSIONS

Specific immune serum is of no value in treating experimental anthrax infections in mice, guinea-pigs or rabbits. Neosalvarsan is also of no value in the treatment of anthrax infection in mice and rabbits according to the technic used. Neosalvarsan is apparently of distinct therapeutic value in guinea-pigs when injected simultaneously with ordinarily fatal doses of anthrax bacilli.