USE OF STREPTOMYCIN TO COMBAT CONTAMINATION OF
TREPONEMA PALLIDUM SUSPENSIONS IN THE TPI TEST*†

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Since the middle of the 16th century when a virus
was suggested as the causative agent of syphilis
by Fracastoro (Pusey, 1933), many organisms have
been labelled as responsible for the disease. For
some time it was thought that syphilis was caused
by a combination of micro-organisms, since the
syphilitic individual was usually suffering from
a mixed infection. This misconception was not
clarified until Schaudinn and Hoffmann (1905)
described the spirochaete alone as the causative
organism, thus ending a 400-year search. The
identification of the specific organism inspired
new enthusiasm, and many publications were
made: Wassermann, Neisser, and Bruck (1906)
described the diagnostic serum-complement reaction;
Noguchi (1911) succeeded in cultivating Treponema
pallidum "in vitro"; Nelson and Mayer (1949) described the Treponema
pallidum Immobilization (TPI) test.

With the advent of the TPI test, it
became necessary to maintain pure
cultures of virulent T. pallidum for use
as the antigen in the test mixture. The
age-old problem of mixed cultures and
contamination presented itself when
many of the sera which were sub-
tected to the laboratory for testing
were contaminated. At first, Corning
ultra-fine, sintered glass filters were
used to sterilize the serum by filtration.
Even though the filtration method was
satisfactory, it was sometimes a lengthy
process and much time had to be spent
in cleaning and sterilizing the filters.
In some instances there was an
insufficient amount of serum to filter,
and new specimens had to be requested
at considerable expense.

It was desirable to find some better
method for sterilizing the contaminated
sera, which would eliminate the long
filtering process, cleaning and steri-
lizing the filters, and the need for re-
placement samples. The logical answer
seemed to be some selective antibiotic
or other agent which would kill the contaminating
organism without noticeable effect on the Treponema
pallidum.

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† The opinions or assertions contained herein are the private ones
of the writers and are not to be construed as official or reflecting the
views of the Navy Department or the naval service at large.
Several of the contaminated sera were streaked on blood agar and the contaminant cultured and identified. In the majority of the cases the intruder was *Bacillus subtilis*. The cultures from the blood agar plates were transferred, using heavy inoculums, to other blood agar plates, and these newly-inoculated plates were then overlaid with "Difco Bacto Sensitivity Disks" containing various concentrations of several antibiotics. They were then incubated at 37°C with readings made at 12, 24, and 48 hrs. Some of the antibiotics which showed the highest toxicity are shown in the Figure. The organism showed high sensitivity to aureomycin, chloramphenicol, dihydrostreptomycin, penicillin, and terramycin, but was only moderately sensitive to polymyxin B, and showed no sensitivity to bacitracin (Table I).

### Table I

Sensitivity of Contaminant to Various Antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Aureomycin</td>
<td>x</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>x</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>x</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>x</td>
</tr>
<tr>
<td>Penicillin</td>
<td>x</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>x</td>
</tr>
<tr>
<td>Terramycin</td>
<td>x</td>
</tr>
</tbody>
</table>

At this point it seemed that any of the five antibiotics for which the organism showed high sensitivity reactions would be acceptable; however, the toxicity of these antibiotics for the treponeme was the next important fact which had to be determined. In order to determine this, 24 tubes were prepared as follows:

(a) 8 tubes containing sterile treponeme suspension;
(b) 8 tubes containing contaminated sera;
(c) 8 tubes containing contaminated treponeme suspension.

Dilutions of the antibiotics indicated in the Figure were added to these tubes. On each of the three types of suspensions, an antibiotic-free control was run. All of the tubes, including the controls, were incubated at 35°C for 18 hrs under anaerobic conditions as in the TPI test. The results are shown in Table II.

### Table II

Treponemal Toxicity of Antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Contaminated Serum Amount of Contaminated</th>
<th>Treponeme Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nil</td>
<td>Moderate</td>
</tr>
<tr>
<td>Aureomycin</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Bacitracin</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Terramycin</td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>Antibiotic-free</td>
<td></td>
<td>Nil</td>
</tr>
</tbody>
</table>

* Averaged percentages

### Conclusions

Dihydrostreptomycin at a concentration of 100 μg killed the contaminating organism without noticeable harmful effect on the viability of the *Treponema pallidum*, or on the residual complement reaction.

This work was completed during the summer of 1952 and has since been used by this laboratory routinely, under the above-mentioned conditions, with excellent results. It is felt that sufficient laboratory trials have been accomplished to prove this procedure to be satisfactory. The TPI laboratory at the Hôpital St. Lazare, Paris, has confirmed our findings.

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### References