Synergy of antibiotic combinations against treponemes

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The purpose of this investigation was to study the effect of various pairs of selected antibiotics on treponemes for possible synergistic activity.

Material and Methods

TREPONEME STRAIN

The Reiter strain of Treponema phagedenis was used as the test organism. Cultures were maintained as described in a previous communication (Abramson and Smibert, 1971a).

PRE-REDUCED MEDIUM

Peptone-yeast extract-glucose-serum (PYGS) medium: Peptone M* 2g.; yeast extract† 1 g.; dextrose 1 g.; agar 0:2 g.; ammonium sulphate 0:05 g.; soluble starch 0:05 g.; L-cysteine HCl hydrate 0:16 g.; sodium bicarbonate 0:5 g.; resazurin solution (25 mg./100 ml.) distilled water 0:4 ml.; salt solution (MgSO4 0:02 g.; CaCl2,2H2O 0:02 g.; K2HPO4 0:1 g.; KH2PO4 0:1 g.; NaCl 0:2 g./100 ml.) 50 ml.; and distilled water 50 ml. The pH of the medium was adjusted to 6·5 before autoclaving and was 6·8 to 7·4 after sterilization.

RABBIT SERUM

Serum was filtered through a 0·45 μm pore diamembrane filter, aseptically dispensed into sterile glass bottles, heat-inactivated at 58° to 60°C. for 4 hrs and stored at −20°C. Sterile rabbit serum was added to tubes of pre-reduced anaerobically sterilized medium at a final concentration of 12 per cent.

Preparation of pre-reduced medium was as described in ‘The Outline of Clinical Methods in Anaerobic Bacteriology’ (Anaerobe Laboratory, 1970), and inoculation of cultures under oxygen-free conditions was accomplished using the V.P.I. Anaerobe Culture System†.

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ANTIBIOTIC COMBINATIONS

A modification of the method described by Eickhoff and others (1970) was used in this investigation. Pairs of antibiotics with bacteriostatic combinations of 0.1 to 10 units or μg./ml. of medium for the Reiter strain of Treponema phagedenis were selected for study. They were penicillin, cephalothin, bacitracin, erythromycin, vancomycin, and tetracycline. The first antibiotic was diluted and pipetted into five series of five tubes each of PYGS medium so that final concentrations were 0.001, 0.01, 0.1, 1, and 10 units or μg./ml. of medium. A second antibiotic at a final concentration of 0.001 units or μg./ml. of medium was pipetted into each tube of a series of five tubes containing the first antibiotic. This procedure was repeated until each one of the dilutions of the second antibiotic, 0.001, 0.01, 0.1, 1, and 10 units or μg./ml., was pipetted into a series of five tubes of medium containing the first antibiotic. All tubes were inoculated with 0.3 ml. of a 24 hrs culture (1 × 10⁶ to 1 × 10⁷ treponemes/ml.) and incubated for 3 days at 37°C. On the third day each culture was transferred to antibiotic-free medium and incubated for an additional 3 days. After incubation each of these cultures was again subcultured into normal medium. All cultures were observed for growth daily for 14 days. Darkfield examinations were made when macroscopic observations of growth were questionable. Each antibiotic was examined for its minimal growth inhibitory and minimal bactericidal level for the Reiter strain of T. phagedenis using concentrations of 0.001, 0.01, 0.1, 1, 10, 100, 500, and 1000 units or μg./ml. of culture medium.

The definitions that we used for antibiotic synergism, additive effect and antagonism are as follows:

1. **Synergism**—The inhibitory or bactericidal concentrations of both antibiotics in combination are less than that of either one of the individual antibiotics;
2. **Additive effect**—The inhibitory or bactericidal concentrations of a combination of antibiotics do not differ from those of either one of the individual antibiotics;
3. **Antagonism**—The inhibitory or bactericidal concentrations of a combination of antibiotics are greater than that of either one of the individual antibiotics.

### Results

Neither synergism nor antagonism was found with the Reiter strain of T. phagedenis at growth inhibitory concentrations of bacitracin-erythromycin, bacitracin-cephalothin, bacitracin-penicillin, bacitracin-tetracycline, bacitracin-vancomycin, erythromycin-cephalothin, erythromycin-penicillin, erythromycin-tetracycline, erythromycin-vancomycin, cephalothin-tetracycline, cephalothin-vancomycin, penicillin-vancomycin, and tetracycline-vancomycin. All combinations were additive at growth inhibitory concentrations.

Synergism was observed with ten antibiotic combinations at bactericidal concentrations (Table). There was a considerable reduction in the amount of antibiotic necessary to kill all the organisms in a culture when compared with the bactericidal levels of the individual antibiotics. Antagonism was not observed with any antibiotic combination at bactericidal concentrations. An additive effect was found at bactericidal levels with combinations of cephalothin-penicillin, cephalothin-vancomycin, penicillin-tetracycline, penicillin-vancomycin, and tetracycline-vancomycin.

### Discussion

We have studied the effect of fifteen selected combinations of antibiotics against the Reiter strain of T. phagedenis. Ten combinations were synergistic at bactericidal concentrations. There was no synergism at antibiotic concentrations that only inhibited growth. A much higher concentration of antibiotics, especially penicillins, is required to be bactericidal to treponemes than is required to inhibit growth (Abramson and Smibert, 1971a, b). Bactericidal concentrations of most antibiotics for cultured treponemes are high and probably cannot be attained in blood, body tissues, and fluids. Growth inhibitory levels of the penicillins, erythromycin, cephalothin, and the tetracyclines can probably be attained in blood serum against the serum requiring treponemes, but can probably not be attained in spinal fluids or aqueous humour. The high doses of penicillin required for adequate treatment of the treponematoses can probably be accounted for by the high concentrations of penicillin needed to be bactericidal. At best, blood levels attained in man are probably only growth-inhibiting for a few hours. Perhaps combinations of those antibiotics suitable for systemic use that were found to be synergistic for cultivable treponemes in vitro should be investigated.

### Table

**Synergy of various combinations of antibiotics against the Reiter strain of T. phagedenis**

<table>
<thead>
<tr>
<th>Antibiotic combinations</th>
<th>Concentration at which no growth appeared in 14 days (μg. or units/ml.)</th>
<th>Synergistic concentrations of combinations at which no growth appeared in 14 days (μg. or units/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X + Y</td>
<td>Control X, Y, X + Y</td>
<td></td>
</tr>
<tr>
<td>ErythromycinK Penicillin G</td>
<td>100, 500, 10</td>
<td>10, 0.001</td>
</tr>
<tr>
<td>Erythromycin Tetracycline</td>
<td>500, 10, 1</td>
<td>0.1, 1</td>
</tr>
<tr>
<td>Erythromycin Vancomycin</td>
<td>100, 10, 1</td>
<td>1, 0.001</td>
</tr>
<tr>
<td>Cephalothin Tetracycline</td>
<td>100, 10, 1</td>
<td>0.001, 1</td>
</tr>
<tr>
<td>Bacitracin Erythromycin</td>
<td>10, 100, 1</td>
<td>0.001, 1</td>
</tr>
<tr>
<td>Bacitracin Cefalothin</td>
<td>10, 100, 1</td>
<td>1, 0.001</td>
</tr>
<tr>
<td>Bacitracin K Penicillin G</td>
<td>10, 500, 1</td>
<td>1, 0.001</td>
</tr>
<tr>
<td>Bacitracin Erythromycin</td>
<td>10, 100, 1</td>
<td>1, 0.001</td>
</tr>
<tr>
<td>Bacitracin Vancomycin</td>
<td>10, 100, 1</td>
<td>1, 0.001</td>
</tr>
<tr>
<td>Erythromycin Cephalothin</td>
<td>500, 100, 1</td>
<td>1, 0.001</td>
</tr>
</tbody>
</table>
Synergism of antibiotic combinations against treponemes

Summary
Combinations of antibiotics that were synergistic at bactericidal concentrations to the Reiter strain of *T. phagedenis* were bacitracin-erythromycin, bacitracin-cephalothin, bacitracin-penicillin, bacitracin-tetracycline, bacitracin-vancomycin, erythromycin-cephalothin, erythromycin-penicillin, erythromycin-tetracycline, erythromycin-vancomycin, and cephalothin-tetracycline. No antagonism was found at bactericidal concentrations for any pairs of antibiotics. There was no synergism or antagonism of any pairs of antibiotics tested at growth inhibitory levels.

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References

Action synergique d’associations d’antibiotiques vis-à-vis des treponèmes

SOMMAIRE

Les associations d’antibiotiques qui ont une action synergique à concentration bactéricide sur la souche Reiter de *T. phagedenis* furent: bacitracine-érythromycine, bacitracine-céphalothine, bacitracine-pénicilline, bacitracine-tétracycline, bacitracine-vancomycine, érythromycine-céphalothine, érythromycine-pénicilline, érythromycine-tétracycline, érythromycine-vancomycine et céphalothine-tétracycline. Aux concentrations bactéricides, on ne constata aucun antagonisme pour aucune paire d’antibiotiques. Au taux bactériostatiques, il n’y eut ni synergie ni antagonisme pour aucune des paires d’antibiotiques examinées.