Dialysis Culture Enables More Accurate Determination of MIC of Benzylpenicillin for *Borrelia burgdorferi* than Does Conventional Procedure

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A constant benzylpenicillin (penicillin G) concentration for determination of the MIC for strains of *Borrelia burgdorferi* was achieved by dialysis culture. The strains were grown in dialysis membrane bags with daily transfer to tubes with freshly added benzylpenicillin. The MICs decreased by one or several dilution steps compared with the conventional procedure.

Clinical manifestations caused by *Borrelia burgdorferi*, such as erythema migrans, meningoradiculitis, and acrodermatitis chronica atrophicans, are usually successfully treated with oral or intravenous penicillin (2,3,16,18,19). In vitro determination of MICs of benzyl penicillin (penicillin G [PenG]) for *B. burgdorferi* have shown variable results (0.003 to 3.0 mg/liter) (4, 9, 12, 13). Because PenG is unstable in solutions, MIC determinations of penicillin for slowly growing bacteria such as *B. burgdorferi* may give misleading results. In one study, the concentration of PenG in the test tube had declined by 75% after 72 h (7). The present study was performed to compare MIC determinations done in the conventional way with penicillin added at the start of culture only with those done with dialysis culture, aimed at keeping the antibiotic concentration constant over the whole incubation period of 7 days. We also measured the decline of the PenG activity in test tubes day by day for 7 days.

**Bacterial strains.** Five strains of *B. burgdorferi* were chosen for this study. One of the strains (ACA-1) was isolated from human skin (1), and three strains (SL42 [10], PKa [14], and DK-6 [11]) were isolated from cerebrospinal fluid. The fifth strain (B31) was isolated from a tick in the United States (5).

**Determination of MIC.** All manipulations were carried out in a laminar flow box to minimize the risk of bacterial contamination. Two methods were used. In the macrodilution method, the five *B. burgdorferi* strains (107 to 109 cells per ml) were added to test tubes containing 10 ml of Barbour-Stoenner-Kelly (BSK) medium (17) with a twofold serial dilution of PenG (4.0 to 0.032 mg/ml) and control tubes without PenG. In the dialysis culture method (15), the bacterial suspensions were enclosed in sealed dialysis membrane bags (Spectra/Por; Spectrum Medical Industries, Houston, Tex. [diameter, 7.5 mm; length, ~2 cm; volume, 0.44 ml/cm; molecular weight cutoff for membrane, 1,000]) in tubes containing 10 ml of the BSK medium with PenG of the same concentrations as for the conventional method. The dialysis bags were transferred every day for 6 days to new tubes containing BSK medium with freshly added PenG. Determinations of MIC were done in single tubes for both methods. All tubes were incubated at 32°C. On day 7, the spirochete cell suspensions were read with a Petroff-Hauser counting chamber with a phase-contrast microscope (×400). The MIC was determined as the lowest concentration in which the number of motile organisms was not greater than the original inoculum.

**Penicillin breakdown in broth.** PenG was added to two test tubes containing BSK medium to a final concentration of 10 μg per ml. A total of 105 *B. burgdorferi* B31 cells per ml were added to one tube. The tubes were incubated at 32°C. Samples were taken every day for 7 days for determination of the PenG concentration by the agar well method with *Staphylococcus aureus* 209 as the indicator strain.

**Inactivation of PenG.** Figure 1 shows the inactivation of PenG in BSK medium. The half-life of PenG was approximately 1.5 days. Addition of spirochetes to the solution did not influence antibiotic activity.

**MIC of PenG for *B. burgdorferi*.** The results of MIC determinations are shown in Table 1. For all strains, the MICs were higher for the conventional method than for the dialysis cul-

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TABLE 1. MIC of PenG for five *B. burgdorferi* strains
determined by dialysis culture and by conventional
macrodilution broth technique

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml) of PenG by:</th>
<th>Dialysis culture</th>
<th>Macrodilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA-1</td>
<td>0.064</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>SL 42</td>
<td>0.032</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>PKa</td>
<td>0.064</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>DK-6</td>
<td>1.0</td>
<td>&gt;4.0</td>
<td></td>
</tr>
<tr>
<td>B31</td>
<td>0.032</td>
<td>&gt;4.0</td>
<td></td>
</tr>
</tbody>
</table>

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REFERENCES