Bactericidal and Sporicidal Activity of a Quaternary Ammonium Resin-Triiodide Complex

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Tests to determine the potential use of a quaternary ammonium resin-triiodide complex as a sterilizing agent showed that it was an ineffective sporicide and that bactericidal activity was impaired by complex milieu.

Taylor, Fina, and Lambert's report (7) of an insoluble quaternary ammonium resin-triiodide complex that was rapidly bactericidal, released bactericide upon demand, and appeared to be relatively nontoxic suggested its use as a sterilizing agent for those types of dental instruments which currently present a sterilization problem (2, 4). Although previously tested triiodides have been considered relatively ineffective germicides (1), Taylor et al (7) showed that when complexed onto an ion exchange resin, the triiodide had bactericidal activity towards water suspensions of five species of nonsporeforming microorganisms. To determine its potential use as a sterilizing agent, sterility testing with both endospores and vegetative cells was performed by the recommended suture loop technique of the Association of Official Analytical Chemists (3, 6), a technique which simulates the submersion methods frequently employed for root canal instrument sterilization, and by testing eluates from cell suspensions passed through resin columns as described by Taylor et al (7).

The triiodide-resin complex was prepared according to Taylor et al (7) by using IONAC A-540 (chloride form; Matheson, Coleman & Bell) with an exchange capacity value of 3.6 meq/g to calculate the amount of triiodide required. Free iodine release was estimated with the cadmium iodide-linear starch reagent (5). For suture loop exposure, 38 g of the resin complex was used as an aqueous slurry contained in a sterilized, covered 50-ml beaker. Clostridium sporogenes, NRRL B1219, and Bacillus subtilis were maintained and propagated as sporulated cultures for testing according to Ortenzio (6), but Bacto fluid thioglycolate medium (Difco #0256-01) was used as suggested by T.J. Czerkowicz (personal communication). All test cultures exhibited excellent sporulation.

The standardized HCl treatment (6) indicated that spore resistance of the B. subtilis cultures was high, but that it was low for C. sporogenes, with only 5% of the cultures yielding growth after treatment. E. coli B was cultured in nutrient broth (Difco Laboratories, Detroit, Mich.) to late log phase (~1 × 10⁹/ml). For testing cells suspended in water, cultures were harvested by centrifugation, resuspended to original volume, and serially diluted in distilled water.

Suture loops were prepared (6) and were either dried after inoculation with the individual spore cultures (6), inoculated immediately before exposure with E. coli broth dilutions in the range of 10⁻¹ to 10⁻⁴ or tested uninoculated. They were submerged individually in the resin-complex slurry for 4- to 6-sec, 0.5-, 1-, 2-, 5-, 10- and 30-min exposure times, followed by the recommended transfer schedule (6). Each of the above groups of sutures was tested repetitively by using three to five sutures per exposure time in each test and two different batches of the triiodide resin complex.

Even after 30 min of exposure to the complex, all of the inoculated suture loops, including those with E. coli, showed growth when subcultured, whereas cultures from the resin-treated uninoculated control sutures were negative for up to 14 days.

As indicated in Table 1, the resin-complex columns were used with and without pretreatment, and cell suspensions in water or in nutrient broth were tested. Three 0.1-ml eluant volumes from each suspension were transferred into thioglycolate (Difco) or nutrient broth, or both, and incubated for up to 21 days. To determine the effect of biological milieu on the triiodide resin complex, 300 ml of either nutrient broth or triple-distilled water was passed through duplicate columns prior to passage of test E. coli dilutions.
yielded positive treated resin, through controls shown by rods, ing resin complex. in water. of 103-1:1

### TABLE 1. Viability following passage through the resin-triiodide complex

<table>
<thead>
<tr>
<th>Resin pretreatmenta</th>
<th>Test material</th>
<th>Inoculum</th>
<th>Cultures tested (no.)</th>
<th>Cultures yielding growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>water</td>
<td>B. subtilisb</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>None</td>
<td>water</td>
<td>C. sporogenec</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>None</td>
<td>water</td>
<td>None</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>nutrient broth</td>
<td>E. coli4</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Water (500 ml)</td>
<td>water</td>
<td>E. coli4</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Nutrient broth (500 ml)</td>
<td>water</td>
<td>E. coli4</td>
<td>9</td>
<td>89</td>
</tr>
</tbody>
</table>

a Passage through resin column prior to test material.

b From 72-hr cultures; dilution factors of 1:1 × 10^2-1:1 × 10^4.

c From 30-hr cultures; dilution factors of 1:1 × 10^1-1:3 × 10^3.

d From 30-hr cultures; dilution factors of 1:1 × 10^2-1:1 × 10^4.

in water. All tests were performed with a minimum of two separately prepared batches of the resin complex.

After passage through columns of unpretreated resin, B. subtilis and C. sporogenes each yielded positive cultures (Table 1) of spore-forming rods, reaffirming the poor sporicidal activity shown by the suture loop test. However, after passage of E. coli water suspensions and water controls through such columns, cultures consistently yielded no growth (Table 1), whereas the control cultures, inoculated from the same suspensions but not exposed to the resin complex, were positive. The bactericidal activity reported by Taylor et al. (9) was therefore confirmed. Significantly, this bactericidal activity was not found under all conditions. It was not observed when the same E. coli cultures were diluted in broth or when columns were pretreated by passage of sterile nutrient broth (Table 1). These findings and the E. coli suture loop results indicate an inactivation of the resin complex. This inactivation may occur by protein absorption to the triiodide, because tests for free iodine release (5) were negative.

Thus, the quaternary ammonium resin-triiodide complexes are unsuitable as sterilizing agents because of inadequate sporicidal activity, and their use as bactericidal agents is limited because of inactivation by biological fluids.

**LITERATURE CITED**