New Water Disinfectant: an Insoluble Quaternary Ammonium Resin-Triiodide Combination that Releases Bactericide on Demand

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Strongly basic anion-exchange resins form stable, water-insoluble combinations with triiodide ions. The combinations have remarkable antibacterial properties: 3.0 \times 10^4 \text{ Escherichia coli} cells per ml were killed when passed through a 3.8-g column of commercially available resin treated with triiodide (volume 4 ml after treatment). In an attempt to deplete the resin-triiodide complex, 1.14 \times 10^4 \text{ E. coli} cells in 15 liters were passed through the column with no significant loss of effectiveness. The antibacterial capabilities of the resin-triiodide columns ranged from 10^6 \text{ Salmonella typhimurium} per ml to 1.1 \times 10^4 \text{ Streptococcus faecalis} per ml. \text{ Staphylococcus aureus} and \text{ Pseudomonas aeruginosa} were also tested and killed at concentrations of 1.8 \times 10^4 and 1.3 \times 10^4 per ml, respectively. The cells were not filtered from the water. They emerged from the column in nonviable form. This was demonstrated by using \text{^{14}C}-labeled bacteria. The irreversible nature of the antibacterial action was revealed when attempts to wash the damaged cells did not restore viability.

A water disinfecting process that releases antibacterial chemicals on demand and leaves no detectable disinfectant in the water is desirable. Nothing was found in the literature regarding such an insoluble compound.

Several forms of iodine, including diatomic iodine, iodof orm, and iodophors, have been recognized as effective germicides (4). Procedures for preparing triiodide solutions are well known (7). Ion-exchange resins often have been used to soften and deionize water but not to disinfect it (8). Producing a water disinfectant by saturating strongly basic anion-exchange resins with triiodide appears to be a new concept.

MATERIALS AND METHODS

Saturation of anion-exchange resins with triiodide. A water slurry of 3.8 g of a strongly basic resin (16 to 30 mesh, chloride or sulfate form) was poured into a funnel attached to a glass tube (inner diameter, 7 to 8 mm) plugged with glass wool. All 3.8-g columns were prepared in that size tubing. The resin was always kept submerged to prevent air pockets from forming. To a 1.0 m aqueous solution of potassium iodide, heated to 80°C, solid iodine was added and dissolved by stirring. To enhance solution, a molar ratio of potassium iodide to iodine of 3.5 to 1.0 was used. The triiodide solution was cooled to room temperature and passed through the column at 15 ml/min. The column was rinsed alternately with distilled water and 1 m aqueous potassium iodide, until the water rinse had no detectable iodine, by using a cadmium iodide-linear starch reagent (6). The potassium iodide rinse minimized the formation of higher polyiodides. After treatment, 3.8 g of resin has a volume of about 4 ml.

The amount of triiodide to be passed through the column was calculated by referring to the exchange capacity of the resin. A 50% excess was added to insure complete saturation. For the calculation, the iodine was considered converted to the triiodide form. The following reaction occurred: \text{I}^- + \text{I}_2 \rightleftharpoons \text{I}_3^-.

Antibacterial properties of the resin-ion complexes. Five different bacterial species were grown at 37°C with shaking for 24 hr in suitable broth. Serial dilutions of the organisms in water were separately passed at 20 ml/min through 3.8 g of resin that had been treated with triiodide. Viable counts of the suspensions were determined before and after they passed through the column. Columns of untreated and iodide-saturated resins served as controls. These organisms were used: \text{Escherichia coli}, \text{Streptococcus faecalis}, \text{Staphylococcus aureus}, \text{Salmonella typhimurium}, and \text{Pseudomonas aeruginosa}. \text{ E. coli} and \text{ S. faecalis} were enumerated by the membrane filter technique (reference 1, p. 610-620), and the others were enumerated by standard pour plate (reference 1, p. 592-593).

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2 M.S. Thesis, Kansas State University, 1970.
Columns (3.8 g) of five different resins were treated with triiodide and tested with *E. coli* as described. The five were Ionac A 540, Amberlite IRA 400, Amberlite IRA 400S, Rexyn 201, and Stamex S 44. A 30-g column of Ionac A 540 was packed in a glass tube (inner diameter, 18 mm) and treated with triiodide. *E. coli* suspensions were passed through this column at 180 rather than 20 ml/min and counted as before.

To test for possible toxic residuals, sterile nutrient broth was passed through a 3.8-g column of Ionac A 540-triiodide and then inoculated with *E. coli*. Numbers were determined as described.

**Experiment to determine whether bacteria are filtered from solution.** *E. coli* cells were grown in a mineral salts medium with 14C-glucose as the only carbon source. The radioactive cells were serially diluted in sterile distilled water and passed through a 30-g column of Rexyn 201-triiodide. Viable counts and radioactivity were determined before and after the cells passed through the column. Radioactivity was measured with a liquid scintillation counter. A similar experiment was conducted with *S. faecalis* grown in Trypticase soy broth containing 14C-glucose.

**Washing cells to restore viability.** A water suspension of 10⁶ *E. coli* per ml was passed through a 30-g column of Rexyn 201-triiodide at 60 ml/min. A small section of glass tubing was immersed in the eluent 5 sec after it was collected. This coated the tubing with bacteria. The piece of tubing was dipped successively into nine nutrient broth tubes to serially wash bacteria free from adhering toxic materials. Growth on incubation in one or more of the nutrient broth tubes would indicate a reversible nature of the reaction.

**RESULTS**

The Ionac A 540-triiodide complex killed high concentrations of all organisms tested (Table 1). The control columns had negligible effect on the viable count. *S. typhimurium* was the most susceptible organism tested.

All types of strong base resins tested demonstrated high antibacterial capabilities against *E. coli*. The Ionac A 540-triiodide complex was the most effective (Table 2).

The 30-g column of Ionac A 540-triiodide killed 1.1 × 10⁶ *E. coli* per ml. This represents nearly a 10-fold increase in effectiveness over that of a 3.8-g column of the same complex.

Results of the other experiments were as follows. The sterile broth that was passed through a resin-triiodide column supported normal growth of *E. coli*. After passing through the column, averages of over 92 and 95%, respectively, of the 14C-labeled *S. faecalis* and *E. coli* were recovered, and the viable count was reduced from 10⁶ and 10⁷ per ml to 0. Washing damaged cells did not restore viability, as there was no growth in any of the nutrient broth tubes. The damage appeared to be immediate and irreversible.

**Table 1. Viable counts of indicated organisms before and after passing through 3.8-g columns of Ionac A 540**

| Organism                      | Viable count per ml | Before passing | After passing |
|-------------------------------|---------------------|---------------|--------------|
| *Salmonella typhimurium*      | 10⁴                 | 0             | 0            |
| *Escherichia coli*            | 3.0 × 10⁴           | 0             | 0            |
| *Pseudomonas aeruginosa*      | 1.3 × 10⁵           | 0             | 0            |
| *Staphylococcus aureus*       | 1.8 × 10⁴           | 0             | 0            |
| *Streptococcus faecalis*      | 1.1 × 10⁵           | 0             | 0            |

* Untreated and iodide-saturated columns had negligible effects on the viable counts.

**Table 2. Antibacterial capabilities of indicated resin-triiodide complexes**

| Brand of resin                         | Description                                      | Viable counts of *E. coli* per ml |
|---------------------------------------|-------------------------------------------------|-----------------------------------|
| Ionac A 540 (Matheson, Coleman & Bell) | Polystyrene quaternary alkyl type, medium porosity, chloride form | 3.0 × 10⁵                        |
| Stamex S 44 (private source)           | Polystyrene tertiary sulfonium type, chloride form | 1.3 × 10⁵                        |
| Rexyn 201 (Fisher Scientific Co.)      | Polystyrene quaternary ammonium type, medium porosity, chloride-sulfate form | 1.0 × 10⁵                        |
| Amberlite IRA 400 (Malinckrodt Chemical Works) | Polystyrene quaternary ammonium type, high porosity, chloride form | 1.4 × 10⁴                        |
| Amberlite IRA 400S (Malinckrodt Chemical Works) | Polystyrene quaternary ammonium type, high porosity, chloride form | 1.2 × 10⁴                        |

* A 3.8-g amount of each resin was saturated with triiodide ion and tested for its ability to kill *E. coli* suspended in water flowing at a rate of 20 ml/min.
DISCUSSION

The negative test in the rinse water by the cadmium iodide-linear starch reagent indicated the presence of fewer than 50 parts per billion of oxidizing agents. The reagent is sensitive to several forms of iodine, including I₂, I₃⁻, IO⁻, IO₃⁻, and IO₄⁻ ions. The aqueous potassium iodide rinse of the column after treatment with triiodide solution helped insure that the iodine present was triiodide and not a higher polyiodide. The resin-triiodide complex was rinsed with such anions as Cl⁻, SO₄²⁻, HCO₃⁻, and NO₃⁻, but none displaced the triiodide ion from the resin.

All types of strongly basic anion-exchange resin-triiodide complexes tested were effective antibacterial agents. It should be noted that the germicidal properties of the triiodide ion, not bound to a resin, have been reported to be negligible compared with diatomic iodine (2, 3, 5, 9). The susceptibility of the test organisms points to broad-spectrum capabilities for the resin-triiodide complex. More organisms are being tested to define the powers of the complex precisely.

When 15 liters of E. coli, 10⁶ cells per ml, was passed through a 3.8-g column of Ionac A 540-I₃ at 20 ml/min, the column killed all of the bacteria (1.14 × 10⁹) without significant loss of effectiveness. The resin, if exhausted, may be recharged by treating with triiodide solution. The use of ¹⁴C-labeled bacteria showed that the cells were not filtered from solution but emerged from the column in nonviable form. The exact mechanism of the antibacterial action is not known but is being investigated. Among the applications for such a column are purification of water for human consumption in houses, hospitals, and in the field, and for industrial cooling water systems in which growth of microorganisms is often a problem. The insolubility and high capacity make this disinfectant ideal for these purposes.

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