THE ANTIBACTERIAL CAPABILITIES OF POLYHALOGENATED ION EXCHANGE RESINS

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Approved by:

Louis R Tsuia
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with love,

to my Mother and Father,

who made my education possible,

and to Nancy, my bride.

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INTRODUCTION

It was thought feasible to try to find a disinfecting process that would release antibacterial chemicals only upon contact, and leave no detectable disinfectant residual in the water. No previous work had been done regarding the antibacterial capabilities of insoluble compounds which release disinfectants upon demand. The possibility that a strong base ion exchange resin-triiodide complex might prove fruitful seemed worthy of investigation.

To prove that such a resin-I_3 complex had potential, several questions would need to be answered. For example, the greatest concentration of bacteria that could be killed would have to be determined. To be really useful such a complex must have a broad spectrum of germicidal action. The question of the mode of action, whether mechanical filtration or physiological disruption occurred, would need investigation. In addition, several non-biological aspects of the resin-I_3 complex would have to be considered. These include inquiries regarding resins of different brands, porosities, and chemical compositions.

The studies were begun with some of these in mind. It should be pointed out that the questions asked above developed with each succeeding positive result.

LITERATURE REVIEW

Several forms of iodine have been recognized as effective disinfectants (3). Studies of the germicidal properties of the triiodide ion report it to have small or negligible bactericidal properties

compared to diatomic iodine (1, 2, 4, 15). The chemistry of the triiodide ion has been widely studied (10), and the ion has been shown to be essentially linear in form (8).

Ion exchange resins have often been used for water conditioning, such as softening and deionizing (14). These resins have not been used to disinfect water. Common types of strong base anion exchange resins produced commercially have quaternary ammonium nitrogen groups as reactive sites. Other strong base resins have active sites (such as tertiary sulfonium ions) (7) with strongly basic properties similar to those of quaternary ammonium groups. These resins were available in the salt form, with the reactive sites weakly bonded to chloride or sulfate ions. Strong base resins have non-reactive polystyrene matrices to which the reactive groups are fixed.

Procedures for preparing polyiodide solutions and salts are well known (9). However, it was an entirely new concept to try to produce a water disinfectant by saturating a strong base anion exchange resin with the triiodide ion.

MATERIALS AND METHODS

Saturation of Ion Exchange Resins with Triiodide Ions

Several techniques for the treatment of ion exchange resins with the triiodide ion were considered. One promising method was treating columns of strongly basic resins with a solution of potassium triiodide (KI_3).

A slurry of four grams of the resin was poured into a funnel attached to a 7-8 mm inside diameter glass tube. The bottom of the

tube was plugged with glass wool to prevent the resin from leaking out. The resin was always kept submerged, thus preventing air pockets from forming.

The triiodide solution was prepared by making a one molar aqueous solution of potassium iodide, and heating it to 80° C. Solid iodine was added and dissolved by stirring the solution until it cooled to room temperature. The following reaction occurred:

1. $KI + I_2 \rightleftharpoons K^+ + I_3^-$

It is also possible for higher polyiodides to form:

2. $I_3^- + I_2 \rightleftharpoons I_5^-$

3. $I_5^- + I_2 \rightleftharpoons I_7^-$

The molar ratio of potassium iodide to iodine was 3.5 to 1.0. The triiodide solution was passed through the column of resin. The amount of triiodide needed was calculated by referring to the exchange capacity of the resin. A fifty per cent excess of triiodide was added to insure complete saturation. For this calculation, the iodine was considered to be converted solely to the triiodide form. The triiodide ions replaced the chloride or sulfate ions on the reactive sites of the resin, thus forming an ion association compound.

A Test for Eluted Iodine in Wash Water

The column was rinsed alternately with distilled water and potassium iodide solution. These rinses removed uncombined iodine and excess iodide, triiodide, potassium, sulfate, and chloride ions from the column. The iodide rinses also insured the conversion of all polyiodide ions that were present to the triiodide form. The

column was washed repeatedly and tested for detectable iodine using a cadmium iodide-linear starch reagent (6).

Antibacterial Properties of Different Resin-Ion Complexes

The germicidal properties of several resin-ion complexes were investigated. Strongly basic resins were tested before and after saturation with different ions. The columns were separately treated with iodine dissolved in ethanol, or aqueous solutions of iodide, triiodide, iododibromide, or permanganate ions. Escherichia coli cultures were serially diluted in sterile water and passed through one of the columns. The I_3^- ion was used to treat several different brands of strong base resins. Samples were taken before and after passing through the columns. Viable counts were determined.

The Germicidal Effect of a Resin-Triiodide Complex on Five Different Organisms

Dilutions of five organisms were separately passed through 3.8 gram columns of the Ionac A 540-I_3 complex at a 20 ml per minute flow rate. E. coli and Streptococcus fecalis were tested because they are indicative of fecal contamination in water (9). Staphylococcus aureus and Salmonella typhimurium were examined to determine the ability of the complex to kill common pathogens. Pseudomonas aeruginosa was used as it is a widely distributed soil and water organism. E. coli and Strep. fecalis were enumerated by the membrane filter technique (13). The other organisms were counted by the standard pour plate method (12).

Bacterial Kill in Nutrient Broth

Serial dilutions of E. coli in nutrient broth were passed through a 3.8 gram Ionac A 540-I_3 column at a flow rate of 20 ml per minute. Sterile broth was also passed through this column, then inoculated with E. coli. The broth was inoculated to test whether or not column passage left a toxic residual in the broth that would inhibit bacterial growth.

Antibacterial Abilities of a Different Size Column

A 30 gram column of Ionac A 540-I_3 was prepared in an 18 mm diameter glass tube. Escherichia coli dilutions were passed through at a 60 ml per minute rate. This was to determine if the different flow rate, column size, and amount of treated resin would result in altered antibacterial capabilities.

A Test for the Total Disinfecting Capacity of a Column

Eleven liters of a suspension of E. coli in water were prepared. The viable counts ranged from 0.4 to 1.7 x 10^5 organisms per ml. This suspension was passed through a 3.8 gram column of Ionac A 540-I_3 in an attempt to exhaust the germicidal capabilities of the column. Samples were taken every 250 ml to test the consistency of the killing action of the resin-I_3 compound.

An Experiment to Determine if Bacteria are Filtered from Solution

E. coli were grown in a mineral salts medium with ^{14}C glucose as the only carbon source. The radioactive cells produced were serially diluted in sterile distilled water, and passed through a

30 gram column of Rexyn 201-I_3^-. Samples were taken before and after passing through the column. The viable counts were determined. Radioactivity of the samples was determined with a liquid scintillation counter (5). A similar experiment was conducted with a Strep. fecalis culture that had been grown in trypticase soy broth enriched with ^{14}C glucose.

Washing Cells to Restore Viability

One million E. coli per ml were passed through a 30 gram column of Rexyn 201-I_3^-. A small section of glass tubing was dipped into the eluent five seconds after it was collected. This piece of tubing was dipped successively into nine nutrient broth tubes. If the bacteria that adhered to the tubing could be washed free of toxic materials, this serial washing would permit growth in one or more of the nutrient broth tubes.

RESULTS

A Test for Eluted Iodine in Wash Water

The cadmium iodide-linear starch reagent detects oxidizing agents as dilute as 50 parts per billion (6). The reagent is sensitive to several forms of iodine, including I_2, I_3^-, IO^-, IO_3^-, and IO_4^- ions. There was no iodine detectable after sufficient washing with aqueous KI and water. Therefore, iodine was present in the eluent in concentrations of less than 50 parts per billion.

Antibacterial Properties of Different Resin-Ion Complexes

The triiodide and iododibromide-treated resins were very effective antibacterial agents (Table 1). However, the resin-IBr_2 complex leaked bromine and was not tested further. The resin treated with iodine dissolved in ethanol released iodine in such great amounts that this combination was not practical. The resins treated with iodide or permanganate ions were ineffective against E. coli. The Ionac A 540-I_3 complex killed 3.0×10^5 E. coli per ml, and the other brands killed concentrations nearly as large (Table 2).

The Germicidal Effect of a Resin-Triiodide Complex on Five Different Organisms

The Ionac A 540-I_3 complex killed significant numbers of all organisms tested (Table 3). Salmonella typhimurium was the most susceptible organism tested, and was killed in concentrations of 1.1×10^6 per ml.

Bacterial Kill in Nutrient Broth

This experiment was done twice. In both cases, the sterile broth that was passed through the column supported normal growth of E. coli. However, variable germicidal results were obtained. The first trial reduced the viable count from 25 per ml to 0 by passing the broth through the column. The second trial showed a viable count of 25 per ml both before and after passing through the column.

Table 1. Antibacterial capabilities of several resin-ion complexes.

Brand of resin	Saturated with	Viable counts of *E. coli* per ml	
		Before passing	After passing
Ionac A 540	Cl^- form	26	7
Ionac A 540	I^-	130	85
Ionac A 540	I_2 in ethanol	4.5×10^5	0
Ionac A 540	I_3^-	3.0×10^5	0
Rexyn 201	IBr_2^-	8.2×10^6	0
Amberlite IRA 400	MnO_4^-	1.0×10^2	0

Table 2. Antibacterial capabilities of several resin-I_3^- complexes.*

Brand of resin	Description	Viable counts of *E. coli* per ml	
		Before passing	After passing
Ionac A 540	polystyrene quaternary alkyl amine type, medium porosity, chloride form	3.0×10^5	0
Stamex S 44	polystyrene tertiary sulfonium type, chloride form	1.3×10^5	0
Rexyn 201	polystyrene alkyl quaternary amine type, medium porosity, chloride-sulfate form	1.0×10^5	0
Amberlite IRA 400	polystyrene quaternary ammonium type, medium porosity, chloride form	1.4×10^4	0
Amberlite IRA 400S	polystyrene quaternary ammonium high porosity, chloride form	1.2×10^4	0

* 3.8 grams of each resin was saturated with I_3^- and tested for its ability to kill *E. coli* suspended in water flowing at a rate of 20 ml per minute.

Table 3. Capacity of an Ionac A 540-I_3 column to kill five types of organisms.*

Organism	Viable count per ml	
	Before passing	After passing
Salmonella typhimurium	1.0×10^6	0
Escherichia coli	3.0×10^5	0
Pseudomonas aeruginosa	1.3×10^5	0
Staphylococcus aureus	1.8×10^4	0
Streptococcus fecalis	1.1×10^4	0

* The organisms were suspended in water and passed through 3.8 gram columns at a flow rate of 20 ml per minute.

Antibacterial Abilities of a Different Size Column

The 30 gram column killed 1.0×10^6 E. coli per ml. This is an increase of nearly a power of ten over the concentration killed by a 3.8 gram column of the same complex.

A Test for the Total Disinfecting Capacity of a Column

The 3.8 gram column of Ionac A 540-I_3 killed 1.14×10^9 E. coli without any apparent loss of efficiency. The consistency of the killing action of the column is shown in Table 4. The two liter sample recorded in Table 4 is typical of the entire eleven liters that were passed through the column. In order to raise the total kill to 10^{10}, it would have been necessary to pass an additional ninety liters of bacteria through the column. It would take the ninety liters seventy-five hours to pass through the column at the 20 ml per minute flow rate. The bacteria would die by normal attrition before the entire volume could pass through the column. Therefore, the experiment was terminated at this point.

An Experiment to Determine if Bacteria are Filtered from Solution

An average of more than 95% of the radioactively labeled E. coli was recovered after passing through the column (Table 5). The viable count per ml of the eluent was zero. The results for the ^{14}C-labeled Strep. fecalis were similar (Table 6).

Washing Cells to Restore Viability

No growth occurred in the nutrient broth tubes where the bacteria were washed. The damage appeared to be immediate, irreversible, and the iodine was combined with the cells in an insoluble relationship.

Table 4. Ability of a resin-I_3 complex to consistently kill bacteria.

Sample taken after (ml)	Dilution	Viable count of *E. coli* per ml*	
		Before passing	After passing
control**	10^8	16	-
control	10^8	18	-
control	10^7	150	-
control	10^7	170	-
250	10^4	1.6×10^5	0
500	10^4	1.6×10^5	0
750	10^4	1.6×10^5	0
1000	10^4	1.6×10^5	0
1250	10^4	1.6×10^5	0
1500	10^4	1.6×10^5	0
1750	10^4	1.6×10^5	0
2000	10^4	1.6×10^5	0

* The water suspensions of *E. coli* were passed through a 3.8 gram column of Ionac A 540-I_3 at 20 ml per minute.

**Controls were taken to determine the viable count per ml before passage.

Table 5. Radioactive counts per ml per minute and viable counts of ^{14}C-labeled E. coli before and after passing through a resin-I_3 column.

Sample taken after (ml)	Counts/min/ml	Radioactivity recovered after passage (%)	Viable counts of E. coli/ml*	
			Before passage	After passage
control	3220	-	3.1×10^5	-
100	2985	92.8	3.1×10^5	0
200	3083	95.8	3.1×10^5	0
300	2970	92.3	3.1×10^5	0
400	3119	96.8	3.1×10^5	0
500	3138	97.4	3.1×10^5	0
600	3085	95.8	3.1×10^5	0

* The E. coli suspension was passed through a 30 gram column of Rexyn 201-I_3 at 60 ml per minute.

Table 6. Radioactive counts per ml per minute and viable counts of ^{14}C-labeled Strep. fecalis before and after passing through a resin-I_3 column.

Sample taken after (ml)	Counts/min/ml	Radioactivity recovered after passage (%)	Viable counts of S. fec./ml*	
			Before passage	After passage
control	831	-	2.8×10^5	-
100	809	97.4	2.8×10^5	0
200	766	92.3	2.8×10^5	0
300	770	92.7	2.8×10^5	0
400	750	90.3	2.8×10^5	0
500	737	88.6	2.8×10^5	0
600	726	87.5	2.8×10^5	0

* The Strep. fecalis suspension was passed through a 30 gram column of Rexyn 201-I_3 at 60 ml per minute.

DISCUSSION

Antibacterial Capabilities

The antibacterial capabilities of resin-triiodide complexes are very high, especially in comparison with the maximum numbers of bacteria allowable in potable water. Water is not considered potable if it has more than one fecal coliform in 100 ml or if the total count exceeds 200 organisms per ml (11). The 3.8 gram columns killed organisms in concentrations over 1,000 times greater than the allowable maximums. The increased capability of the 30 gram column to kill *E. coli* indicates that even greater killing ability may be obtained by using still larger or more intricately designed columns.

Possible Structure and Mechanism

The proposed structure involves the linear triiodide ion bonded to a quaternary ammonium group of the resin (Plate I). This structure indicated a possible mechanism of antibacterial action. It was thought that the linear triiodide ion could become polarized enough to have a partial positive charge on the terminal iodine atom (Plate I). This partial positive charge could have been sufficient to attract the negatively charged bacteria close to it. In close contact, it was possible that one or two iodine atoms may have separated from the resin and attached to the bacteria. If this happened often enough, the bacteria would be killed by a mechanism similar to the one that kills bacteria treated with diatomic iodine.

EXPLANATION OF PLATE I

Fig. 1. A strong base resin-triiodide complex.

Fig. 2. The resin-triiodide complex with the triiodide ion polarized.

PLATE I

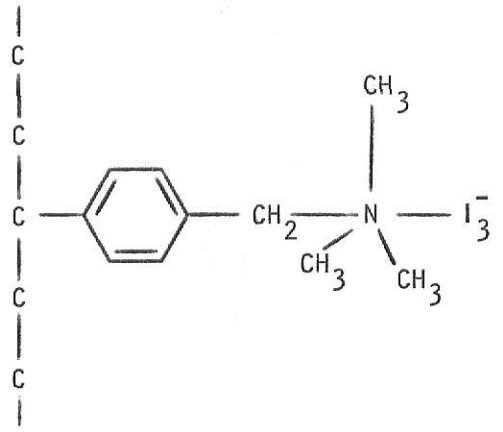

Fig. 1

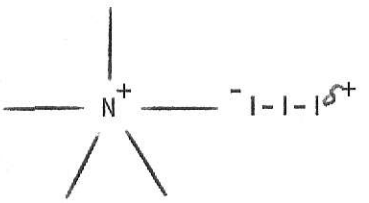

Fig. 2

Possible Future Applications

The insolubility of the resin-ion complexes indicates that they would be stable enough for prolonged use as antibacterial agents. Because of the stability and irreversible germicidal abilities of this complex, several commercial applications may be possible. The resin-I_3 compound could be used for the treatment of both rural and municipal water supplies. The military could adapt the resin-I_3 complex to water treatment in field operations. Columns could be used by hospitals to sterilize water without autoclaving. Space travelers may use columns as a final treatment for their drinking water. There are undoubtedly several other applications possible.

SUMMARY

A method was presented for disinfecting water without leaving a detectable chemical residual. Strong base anion exchange resins form a stable complex with the triiodide ion. These resin-ion complexes kill bacteria in large numbers. The killing capabilities of the complex were determined for five different organisms, and ranged from 1.0×10^6. Salmonella typhimurium per ml to 1.1×10^4 Streptococcus fecalis per ml killed. It was shown with ^{14}C-labeled cells that bacteria were not filtered from solution, but passed on through the column in a non-viable form. The viability of damaged cells was not restored by washing them in nutrient broth. It was proposed that atoms of iodine were removed from the resin by the bacteria, and that these atoms killed in a manner possibly similar to that of diatomic iodine.

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BIBLIOGRAPHY

1. Anson, M. L., and W. M. Stanley. 1941. Some effects of iodine and other reagents on the structure and activity of tobacco mosaic virus. J. Gen. Physiol. 24:679-689.

2. Carroll, B. 1955. The relative germicidal activity of triiodide and diatomic iodine. J. Bacteriol. 69:413-417.

3. Gershenfeld, L. Iodine. In Disinfection, Sterilization, and Preservation, by C. A. Lawrence and S. S. Block. Lea and Febiger, Philadelphia. 1968. pp. 329-342.

4. Hsu, Y., S. Nomura, and C. W. Kruse. 1965. Some bactericidal and virucidal properties of iodine not affecting infectious RNA and DNA. Am. J. Epidemiol. 82:317-328.

5. Keith, C. L. 1963. Development of an improved in vivo artificial rumen (VIVAR) technique and some of its properties. M. S. Thesis. Kansas State University.

6. Lambert, J. L., and F. Zitomer. 1963. Simplified preparation of cadmium iodide-linear starch reagent for colorimetric iodimetry. Anal. Chem. 35:405.

7. Lindenbaum, S., G. E. Boyd, and G. E. Myers. 1958. Properties of a new strong base anion exchanger containing structurally-bound tertiary sulfonium cations. J. Phys. Chem. 62:995-999.

8. Popov, A. I. Polyhalogen complex ions. In Halogen Chemistry. Vol. 1. by V. Gutmann. Academic Press, London and New York. 1967. pp. 229-233.

9. Popov, A. I., and R. E. Buckles. Polyhalogen complex salts. In Inorganic Synthesis. Vol. 5. Edited by T. Moeller. McGraw-Hill, New York. 1957. 167-175.

10. Sharpe, A. G. 1950. Interhalogen coumpounds and polyhalides. Quart. Rev. 4:118-120.

11. Standard methods for the examination of water and wastewater. Twelfth edition. American Public Health Association, New York. 1965. pp. 569-571.

12. Ibid. pp. 592-593.

13. Ibid. pp. 610-620.

14. Wheaton, R. M., and A. H. Seamster. Ion Exchange. In Encyclopedia of Chemical Technology. Vol. 11, second edition. John Wiley and Sons, New York. 1966. pp. 871-899.

15. Wyss, O., and F. B. Strandskov. 1945. The germicidal action of iodine. Arch. Biochem. 6:261-268.

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Strongly basic anion exchange resins (quaternary ammonium or tertiary sulfonium types, chloride or sulfate forms) form a stable, water-insoluble complex with the triiodide (I_3^-) ion. The resin-I_3 complex has remarkable antibacterial properties. A 3.8 gram column of the complex kills 3.0×10^5 Escherichia coli per ml, and 1.14×10^9 total without any noticeable loss of efficiency. These bacterial concentrations are far above those allowable in potable water. Work reported here shows that bacteria were not filtered from the water by passing through the column. This was demonstrated using ^{14}C-labeled bacteria. The irreversible nature of the antibacterial action was revealed when washing the damaged cells did not restore viability.

The antibacterial capabilities of the resin-I_3 complex against four other organisms ranged from 1.0×10^6 Salmonella typhimurium per ml to 1.1×10^4 Streptococcus fecalis per ml killed. Staphylococcus aureus and Pseudomonas aeruginosa were also tested and killed at concentrations of 1.8×10^4 and 1.3×10^5 per ml, respectively.