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Characterization of the effect of aluminum metal on poliovirus

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SUMMARY

Metallic aluminum has been studied for possible use in conjunction with or as an alternative to conventional soluble disinfectants as applied to drinking and wastewater. Acid-washed aluminum was incubated with [^{35}S]methionine-labeled poliovirus type 1 (LSc) and the counts per min (cpm)/plaque-forming units (pfu) ratio was determined. After 2 h, only 0.013% of the cpm remained in solution, indicating viral adsorption onto the surface of the aluminum. After 76 h, 93% of the cpm returned to solution, while infectivity dropped from 2.2×10^7 pfu/ml to undetectable levels. This suggests that infectious viruses were adsorbed onto the aluminum surface and released from the surface of the aluminum as non-infectious particles. Analysis by electron microscopy, cesium chloride gradient and polyacrylamide gel electrophoresis indicates that either dissociation or destruction of the viral capsid proteins occurs during incubation with aluminum, which results in viral inactivation.

INTRODUCTION

Current sewage treatment processes may not always completely disinfect pathogenic human enteric viruses [5,9]. As the infective dose of many viruses may be very low [11] and viruses can survive and be transported in surface and subsurface waters [1,3], the release of any viruses into the environment may pose a health risk to individuals who contact the water at some other place and time.

Commonly used soluble disinfectants, such as chlorine, bromine and iodine, must be dosed into the water to be treated. This requires monitoring of the disinfectant concentration and determination of optimal quantities of the disinfectant to add to the treatment system. Failure to maintain adequate disinfectant residual due to poor monitoring or dosing may result in incomplete treatment of the water and allow pathogenic viruses, such as hepatitis A virus, poliovirus, coxsackievirus, echovirus and norwalk virus, to escape disinfection.

This research investigated the mechanisms of the virucidal activity of metallic aluminum [10], an insoluble contact disinfectant, for possible point-of-use application or use in conjunction with or as an alternative to commonly used conventional soluble disinfectants.

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METHODS AND MATERIALS

Virus. Poliovirus type 1 (LSc), originally obtained from the American Type Culture Collection (Rockville, MD) was used. The plaque-forming unit (pfu) method of assay similar to that described by Melnick et al. [6] was used to determine the infective concentration of poliovirus.

Preparation of radioactively labeled poliovirus. Complete Buffalo Green Monkey kidney monolayers in 150 cm^2 tissue culture flasks were starved of the amino acid methionine by replacing growth medium with serum-free, methionine-deficient modified Eagle's medium (Irvine Scientific, Santa Ana, CA) for 2 h before infecting the monolayer with stock poliovirus to a multiplicity of infection of 30 pfu per cell. The infection was allowed to proceed for 2 h to ensure that host cell protein synthesis had stopped before adding 10 μl [^{35}S]methionine per flask (specific activity, 1108 Ci/mmol; NEG-009T, DuPont, Claremont, CA). This enabled incorporation of the radioactive label into the newly produced virus particles.

The infection was allowed to continue until almost complete destruction of the monolayers was noted. Labeled viruses were purified by freezing and thawing the cells three times, followed by two freon extractions (1,1,2-trichlorotrifluoroethane, Aldrich Chemical Co., Milwaukee, WI). Cell debris was then pelleted from the virus-containing aqueous phase of the freon extraction by spinning for 10 min at 32 560 $\times$ g in a model L8-70 ultracentrifuge (Beckman Instruments, Inc., Irvine, CA) using an SW25.1 rotor (Beckman Instruments, Inc., Irvine, CA). The labeled virus was then pelleted in the same rotor at 90 400 $\times$ g for 2.4 h. The pelleted virus was resuspended in Tris (Sigma Chemical Co., St. Louis, MO) buffered saline and freon-extracted again before banding in cesium chloride (CABOT, Revere, PA), adjusted to 1.34 g/ml density, using a TST 60.4 rotor at 200 700 $\times$ g for 20 h (DuPont Co., Sorvall Products, Wilmington, DE). Fractions were collected by bottom puncture, counted in a scintillation counter and dialyzed against Tris-buffered saline at 4°C for 24 h before being titered and frozen until use.

Liquid scintillation counting. Five milliliters scintillation cocktail (Formula 963, NEN Research Products, Boston, MA) were dispensed into plastic scintillation vials and radioactive [^{35}S]methionine-labeled poliovirus was counted on an LS1800 scintillation counter (Beckman Instruments, Inc., Irvine, CA) until a 2 sigma value of 5% had been reached.

Metallic aluminum. Powdered, metallic aluminum (particle size 10–50 μm, Stansi Scientific Co., Chicago, IL) was first rinsed in 1 N HCl for 2 min, rinsed in 1 N NaOH for 2 min, washed three times in distilled water, and baked for 2 h at 80°C in a vacuum oven prior to use.

Transmission electron microscopy. Monodispersed poliovirus purified by cesium chloride gradient was used for determination of the particle/pfu ratio. Virus samples were split; half were incubated with aluminum and half were incubated without aluminum in microcentrifuge tubes for 76 h. The supernatant fluids were then prepared for transmission electron microscopy by mixing samples with polystyrene microspheres (79 nm, Polybead-Polystyrene Microspheres, Polysciences, Inc., Warrington, PA) according to the procedure described by Miller [7]. Next, 4% phosphotungstic acid and serum bovine albumin were added and the mixture was nebulized onto 300 mesh carbon-only grids (Ted Pella, Inc., Tustin, CA). Grids were examined using an H500 transmission electron microscope (Hitachi Scientific Instruments, Mountainview, CA). All virus particles and beads were counted in each field observed until 100 viruses were noted. The number of virus particles per ml was calculated using the following equation:

$$\text{Concentration of virus} = \frac{\text{observed No. of virus particles}}{\text{observed No. of beads}} \times \text{concentration of beads}$$

Effect of time on virus removal. In order to study the effect of time on the removal of poliovirus, 0.1 g aluminum was incubated with 10^4–10^6 pfu/ml virus diluted in dechlorinated Tucson tapwater (pH 7.91, nephelometric turbidity units 0.2, total dissolved solids 200 mg/l) and shaken for 6 h at 22°C.

At selected time points aliquots of the supernatant fluid were assayed for virus infectivity.

Effect of aluminum on the viability and integrity of poliovirus. In order to determine the effect of aluminum on the infectivity and recovery of labeled poliovirus, the labeled virus was incubated with aluminum and shaken for 76 h. At preselected time points aliquots of the supernatant fluid were analyzed for pfu and cpm. At the end of the 76 h incubation period the remaining supernatant fluid was analyzed by electron microscopy, cesium chloride gradient or gradient polyacrylamide gel electrophoresis.

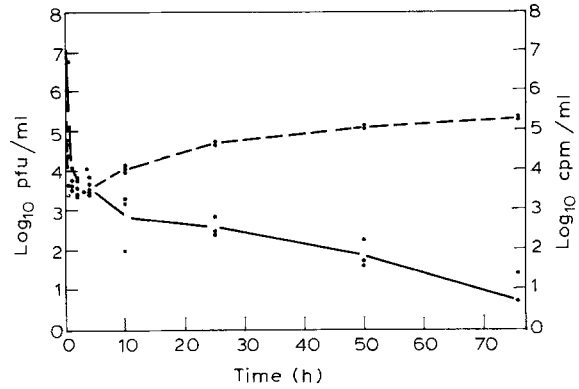

Fig. 2. Effect of aluminum (100 mg) on the cpm and pfu of [^{35}S]methionine-labeled poliovirus. ———, pfu; – – –, cpm.

RESULTS

Fig. 1 shows the effect of increasing time on the removal of poliovirus when incubated with aluminum. Data indicate that poliovirus removal increased with an increase in contact time between the virus and the aluminum.

In order to determine the fate of viruses incubated with aluminum, experiments were conducted using poliovirus labeled with [^{35}S]methionine. Over a period of 76 h the cpm and pfu of the supernatant fluid containing purified, labeled poliovirus incubated with aluminum were measured. A sharp, initial decrease in pfu from 2.2×10^7 pfu/ml to 3.9×10^3 pfu/ml occurred within the first 2 h of incubation. A more gradual decrease in pfu occurred during the remainder of the experiment. The cpm showed a similar sharp, initial decrease which paralleled the decrease noted for the pfu. After 2 h the cpm in the supernatant fluid began to rise until 93% of the initial cpm were back in the supernatant fluid (Fig. 2).

An attempt was made to elute infectious poliovirus from the surface of the aluminum after 76 h of incubation with the aluminum. Beef extract (3%, pH 9.0) was unable to elute infectious virus from the surface of the aluminum; however, some residual radioactive label was recovered (data not shown).

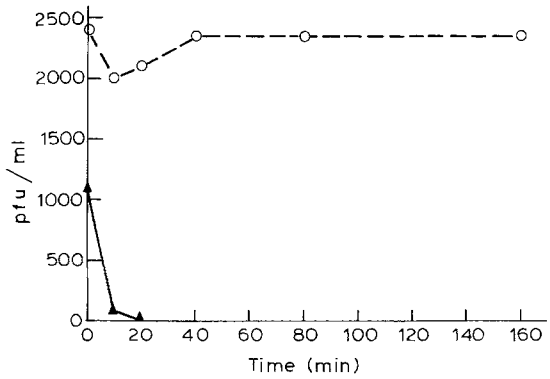

Fig. 1. Effect of time on the removal of poliovirus incubated with aluminum (500 mg). ○, control; ▲, aluminum.

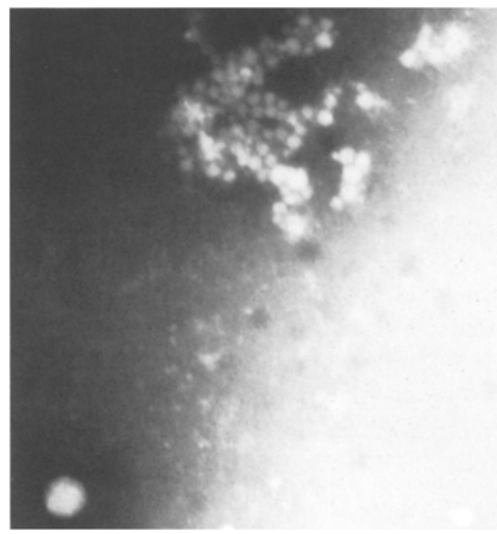

Fig. 3. Electron micrograph of poliovirus after incubation with aluminum. Large object is 79 nm bead.

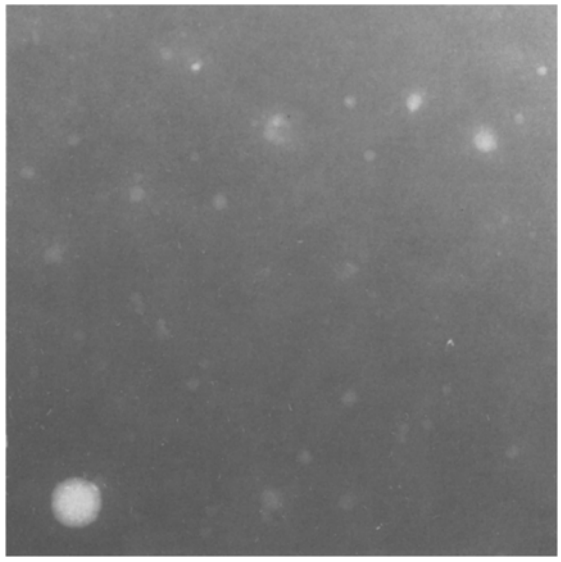

Fig. 4. Electron micrograph of freon-extracted poliovirus after incubation with aluminum. Large object is 79 nm bead.

Transmission electron micrographs indicate that the stock poliovirus used in experiments was monodispersed. Fig. 3 shows supernatant samples of poliovirus after exposure for 76 h to aluminum. Clumps of viruses and viral fragments are noted. Freon was used to disrupt the viral clumps. The dispersed clumps were found to be composed of particles ranging in size from apparently full-sized virions to particles of various smaller sizes (Fig. 4), none of which were found to be infectious by standard tissue culture procedures. The particle/pfu ratio for stock poliovirus was determined as 91:58. Ratios for poliovirus after incubation with aluminum and the freon extraction of poliovirus after incubation could not be determined, as clumping and viral fragments were observed.

Fig. 5 shows a cesium chloride gradient of [^{35}S]methionine-labeled poliovirus after 76 h incubation with aluminum compared with a control (poliovirus incubated for 76 h with no aluminum). The control had a peak representing intact virus, while the viral peak from the experimental tube was greatly reduced. A large peak of radioactive counts was found at the top of the gradient from the experimental tube.

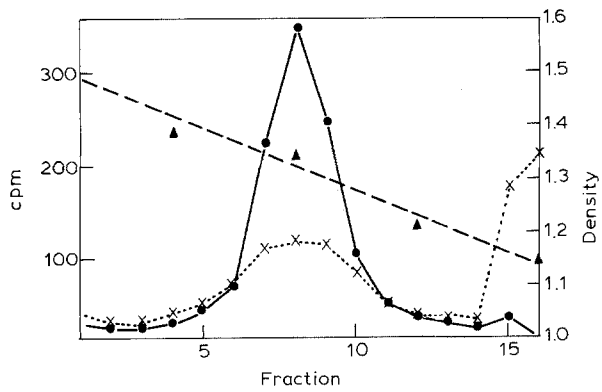

Fig. 5. Effect of aluminum (100 mg) on the integrity of [^{35}S]methionine-labeled poliovirus after 76 h of incubation, as determined by cesium chloride gradient. Fraction 1 = bottom, fraction 16 = top of gradient. ●, control; ×, experimental; ▲, density.

DISCUSSION

Because viruses do not metabolize outside a host cell, are of an extremely small size (20–200 nm) and have a low infectious dose, it is especially difficult to inactivate or remove them from water being treated. Individuals contacting improperly treated water or who use untreated water may be subject to an increased health risk. This study investigated the mechanism of virus inactivation by metallic aluminum in order that its potential for use in water treatment be better understood.

Increased contact time between poliovirus and aluminum resulted in increased removal of poliovirus. This suggests that contact time or available reactive surface area of the aluminum may be a consideration if aluminum is to be designed into a water treatment system.

To investigate the action of aluminum on virus inactivation, labeled poliovirus was incubated with aluminum. As the pfu of the supernatant fluid decreased, it was paralleled by a similar reduction in cpm. This suggests that in order for viruses to be inactivated they must leave the supernatant fluid and contact the aluminum. It was also found that the cpm of the supernatant fluid began to increase, following the initial decrease, after 4 h. This suggests that aluminum–virus contact was made, in-

activation occurred, as indicated by the continual decrease in pfu, and finally inactivated viruses were released from the surface of the aluminum and reappeared in the supernatant fluid as non-infectious cpm.

Electron micrographs indicate that inactivated viruses released from the surface of the aluminum may occur either as viral fragments or in clumps. This is further substantiated by cesium chloride gradient analysis in which a peak of radioactive counts appears at the top of the gradient. This peak represents [^{35}S]methionine-labeled protein fragments or dissociated viral capsid proteins as a result of the incubation of poliovirus with aluminum.

Studies on the three-dimensional configuration of the viral capsid proteins of poliovirus [4] indicate that VP1 occupies the largest portion of the surface, followed by VP2 and finally VP3. This suggests that the order of availability to make contact with the surface of aluminum is as follows: VP1 > VP2 > VP3, while the internal VP4 is not initially able to contact the aluminum.

Murray [8] found that aluminum degrades the RNA of intact poliovirus. This suggests that the residence time of poliovirus on the surface of aluminum is sufficiently long to react with the viral capsid proteins and expose the RNA to the aluminum. It is therefore suggested that VP4 may, in all likelihood, be exposed to the surface of the aluminum after the initial aluminum–virus contact is made and the viral capsid dissociated or destroyed.

The protein–aluminum interaction may be due, initially, to electrostatic attraction (physisorption) between the negatively charged virion and the positively charged aluminum surface. The aluminum surface will be expected to consist of a layer of aluminum oxide or coordinated hydroxyl groups as a result of the interaction of the aluminum with the aqueous phase. Nucleophilic attack of the peptide backbone due to coordinated hydroxide radicals may result in cleavage and protein fragment generation (Fig. 6) as more of the surface of a virus contacts the aluminum surface. The release of viral protein from the surface of the aluminum may occur as bonds are cleaved and sections of the peptide backbone diffuse into the supernatant fluid. Any

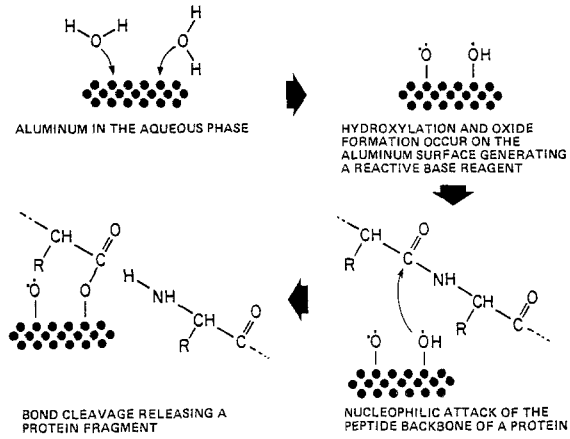

Fig. 6. Possible effect of aluminum on the peptide backbone of the capsid proteins of poliovirus.

portion of a capsid protein that remains on the surface of the aluminum will be expected to reduce the efficiency of virus inactivation of the aluminum by occupying virus adsorption sites.

Capsid dissociation rather than capsid degradation may be the mode of virus inactivation. Hogle et al. [4] state that the amino termini of the capsid proteins of poliovirus are wrapped around each other forming the capsid. The electrostatic attraction between the capsid proteins and the surface of aluminum may be sufficient to allow the capsid to dissociate by overcoming the forces holding the capsid proteins together. Release of the viral capsid proteins from the surface of aluminum is not complete, suggesting that the efficiency of virus inactivation will decrease with time.

The health effects of aluminum in humans are not well defined. It is therefore imperative to minimize or prevent the risk of release of aluminum into the environment or into waters being treated with aluminum. As aluminum ions predominate below pH 4.7 [2], it is essential to ensure that water at or below that pH does not contact the aluminum used in water treatment.

Replacement of the aluminum or cleaning of the aluminum will be required periodically in order to ensure that virus inactivation continues. Possible uses of aluminum or materials with similar activity may include point-of-use applications: household taps, septic tanks, or water storage tanks.

ACKNOWLEDGEMENTS

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