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Disinfection of Bacteria Attached to Granular Activated Carbon

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Heterotrophic plate count bacteria, coliform organisms, and pathogenic microorganisms attached to granular activated carbon particles were examined for their susceptibility to chlorine disinfection. When these bacteria were grown on carbon particles and then disinfected with 2.0 mg of chlorine per liter (1.4 to 1.6 mg of free chlorine residual per liter after 1 h) for 1 h, no significant decrease in viable counts was observed. Washed cells attached to the surface of granular activated carbon particles showed similar resistance to chlorine, but a progressive increase in sublethal injury was found. Observations made by scanning electron microscope indicated that granular activated carbon was colonized by bacteria which grow in cracks and crevices and are coated by an extracellular slime layer. These data suggest a possible mechanism by which treatment and disinfection barriers can be penetrated and pathogenic bacteria may enter drinking water supplies.

Information available indicated that there was a decrease in the number of waterborne disease outbreaks in the United States during the period from 1938 to 1955. However, during the last decade there has been a pronounced increase in both the number of cases and outbreaks of waterborne disease reported by the Centers for Disease Control in Atlanta, Ga. (16). Nineteen outbreaks were reported in 1971, involving 5,182 individuals; 32 outbreaks of waterborne disease with 11,435 cases were reported in 1978 (8, 9). More recent data also support this trend. During the period from 1971 to 1978, 224 waterborne outbreaks of disease were reported in the United States. Two deaths and 48,193 illnesses were associated with these outbreaks in which water was epidemiologically implicated as the vehicle of transmission (9). At the same time, some experts believe that as many as 90% of the outbreaks are unreported, since many people fail to associate their illness with contaminated drinking water (23). This increase might only be an apparent result of improved reporting or a real phenomenon resulting from the overloading of U.S. treatment plants with source water of increasingly lower quality (16). In 1978, treatment deficiencies such as inadequate filtration and interruption of disinfection resulted in 33% of the outbreaks and 54% of the illness in community systems (9). These findings indicate that health problems in this country are increasingly associated with drinking water processed by treatment facilities.

Because of poorer source water in many localities and a need to reduce organic contamination of drinking water that results in taste and odor problems as well as high levels of trihalomethanes, many water systems employ powdered or granular activated carbon (GAC) in the treatment process (2, 4, 11, 14, 17, 20).

A problem arises when carbon particles penetrate treatment filters (19, 21) or when bacteria are sloughed or sheared off filter beds by hydraulic forces and enter effluent waters (22a). Recently we presented evidence that water treated by GAC or powdered activated carbon contains carbon particles in finished effluents (Abstr. Annu. Meet. Am. Soc. Microbiol. 1984. N33, p. 184). A primary concern stemming from this observation is whether bacteria attached to carbon particles can survive chlorination better than nonsorbed cells, since much of our current knowledge of disinfection is based solely on the latter. The present study examines the disinfection efficiency of carbon-associated bacteria. The results show that bacteria attached to carbon particles are highly resistant to chlorination. These results are also true for several pathogenic microorganisms.

MATERIALS AND METHODS

Organisms. The *Escherichia coli* strain used in some of the studies was isolated by membrane filtration from the East Gallatin River near Bozeman, Mont. The isolate was identified and maintained as previously described (5). *Salmonella typhimurium* was obtained from the Wisconsin State Laboratory of Hygiene and was a fecal isolate from an outbreak in 1983. The *Yersinia enterocolitica* (O:8) strain was a gift from Donald A. Schiemann, Montana State University. The *Shigella sonnei* isolate was from the culture collection of the Department of Microbiology at Montana State University.

Attachment procedures. Survival of attached and unattached bacteria during chlorination was examined by three different attachment procedures. (i) A core sample of GAC containing naturally occurring bacteria was obtained from a water treatment plant in Kentucky. The sample was maintained in the laboratory by passing chlorinated drinking water (average temperature, 10°C; free chlorine residual, 1.2 mg/liter; total organic carbon, 4 mg/liter) through a column at a rate of 0.5 to 1.0 gallons/ft^2 (ca. 0.203 to 0.406 liters/cm^2) of carbon per min. Unattached heterotrophic plate count organisms were prepared by inoculating a flask of plate count broth with a few carbon particles. The culture was incubated at 35°C for 48 h, at which time the cells were harvested and washed with reagent-grade water. (ii) The second attachment procedure consisted of growing *E. coli* in a dilute solution of TLY broth (tryptic soy broth with 10% lactose and 3% yeast extract) (1:100 dilution) containing several grams of activated carbon. Cultures were incubated at room temperature for 48 h. This preparation resulted in a biofilm of bacteria and organic material embedded in an extracellular polymer matrix on the carbon surface. (iii) The last attachment procedure consisted of allowing washed *E. coli* cells to attach to virgin GAC for 20 min at 35°C. This preparation resulted in an attachment efficiency of ca. 20%. Cells in this procedure had no time to produce extracellular material and were essentially "naked" on the carbon surface. The two attachment procedures (ii and iii) allowed us to evaluate the role of the activated carbon surface and extracellular polymers during disinfection.

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Chlorination procedure. A stock solution of commercial chlorine bleach (Serco bleach; S. E. Rycoff Co., Los Angeles, Calif.) was prepared daily. Attached and unattached *E. coli* (ca. 10^6 CFU/ml; 0.1 g of GAC in 100 ml of chlorine demand-free water) were chlorinated with an initial concentration of 2.0 mg of free chlorine per liter. Chlorinated solutions were incubated in the dark at 4°C for up to 1 h. At timed intervals free chlorine residuals were measured by amperometric titration (Fischer and Porter titrator). After chlorine determinations, sodium thiosulfate (0.008% final concentration [1]) was added to neutralize any chlorine remaining in the solution.

Homogenization procedures. Bacteria were desorbed from carbon particles by procedures described elsewhere (A. K. Camper, M. W. LeChevallier, S. C. Broadaway, and G. A. McFeters, submitted for publication). Briefly, carbon samples were homogenized for 3 min at 16,000 rpm with a mixture of zwittergent 3-12 (10^{-6} M), ethylene glycol-bis-(β-aminoethyl ether) *N,N*-tetraacetic acid (10^{-3} M), Tris buffer (0.01 M; pH 7.0), and peptone (0.1%). During homogenization, samples were maintained at 4°C by immersion in an ice bath. All viable counts were performed by the spread plate method (1).

Injury. Measurements of cellular injury were used to determine whether sublethal concentrations of chlorine were interacting with attached cells. Injury was measured by the difference on TLY and TLY-D media as previously published (15).

Scanning electron microscopy. Carbon samples were prepared for scanning electron microscopy by mounting carbon granules on a copper support with colloidal graphite (Ted Pella Inc., Tustin, Calif.) and were critical point dried in a Sorvall critical point drying system. Samples were shadow casted with a platinum coating and examined with a JEOL model JEM-100 CX scanning-transmission electron microscope.

RESULTS

Bacteria attached to activated carbon are highly resistant to disinfection by chlorine. Carbon particles from an operating drinking water treatment plant that were colonized with naturally occurring heterotrophic plate count bacteria were treated with 2.0 mg of free chlorine per liter for time periods ranging up to 1 h. A free chlorine residual of 1.7 mg/liter was still present in solution after a contact time of 1 h. No significant decrease in viable counts was observed during this time interval (Fig. 1). The viability of unattached cells, however, decreased by more than 5 log units within 5 min. The data demonstrated that bacteria attached to activated carbon particles were able to completely survive conditions normally encountered in chlorine disinfection during the treatment of drinking water.

Examination of the carbon particles by scanning electron microscopy revealed why these bacteria showed such remarkable persistence. Figure 2 demonstrates that carbon was colonized by bacteria which grow in cracks and crevices and are coated by an extracellular slime layer. The figure also indicates that protrusions were relatively clear of bacteria and microscopically visible organic material, probably because the carbon has been in service for several years and was worn on the edges. The micrographs also show that bacteria were intertwined in the pores of the activated carbon. It is readily understandable why chlorine has little effect on these organisms, because bacteria in the cracks, crevices, and pores of the carbon may never come into contact with the chlorine.

A similar series of experiments were performed with *E. coli* grown on activated carbon and washed cells attached to the GAC surface. The results of these experiments demonstrated the influence of an activated carbon substratum alone as well as the combined effect of extracellular polymers and organic material coating the carbon particles on the disinfection efficiency of hypochlorite. Figure 3 shows the effect of chlorine on *E. coli* grown on GAC. When this preparation was disinfected with 2.0 mg of chlorine per liter (1.4 to 1.6 mg of free residual per liter after 1 h) for 1 h, no significant decrease in viability was observed. Cultures were also plated on TLY and TLY-D media to observe whether attached cells were injured. The rationale behind this experiment was to determine whether chlorine was coming into contact with the attached cells. If this occurred and the cells did not die, they might be injured. No injury was observed, indicating that chlorine was not penetrating the biofilm after 1 h of contact (Fig. 3). However, unattached cell densities dropped 3 log units within 10 min.

Scanning electron microscopy was used to help explain the chlorine resistance that *E. coli* gains when grown on GAC. Figure 4 shows such an *E. coli* cell covered with an extracellular polymer layer (shriveled due to dehydration). The micrographs also show cells growing in a crack on a GAC particle. Both of these mechanisms allow *E. coli* to be highly resistant to chlorination.

Washed *E. coli* cells were attached to GAC for 20 min before disinfection. In this preparation there was insufficient time for extracellular polymer production. This experiment, therefore, indicated the effect of chlorine on cells attached to GAC with little or no extracellular polymeric material (Fig. 5). When this preparation was chlorinated (2.0 mg of free chlorine per liter for 1 h; 1.4 to 1.7 mg of free residual per liter after 1 h), no significant decrease in viability of attached cells was again observed (Fig. 6). This indicated that attach-

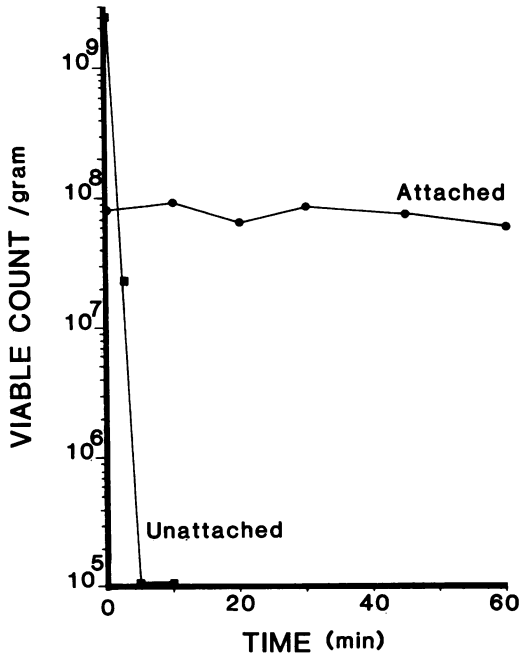

FIG. 1. Survival of naturally occurring heterotrophic plate count bacteria exposed to 2.0 mg of chlorine per liter for 1 h (free chlorine residual after 1 h was 1.7 mg/liter).

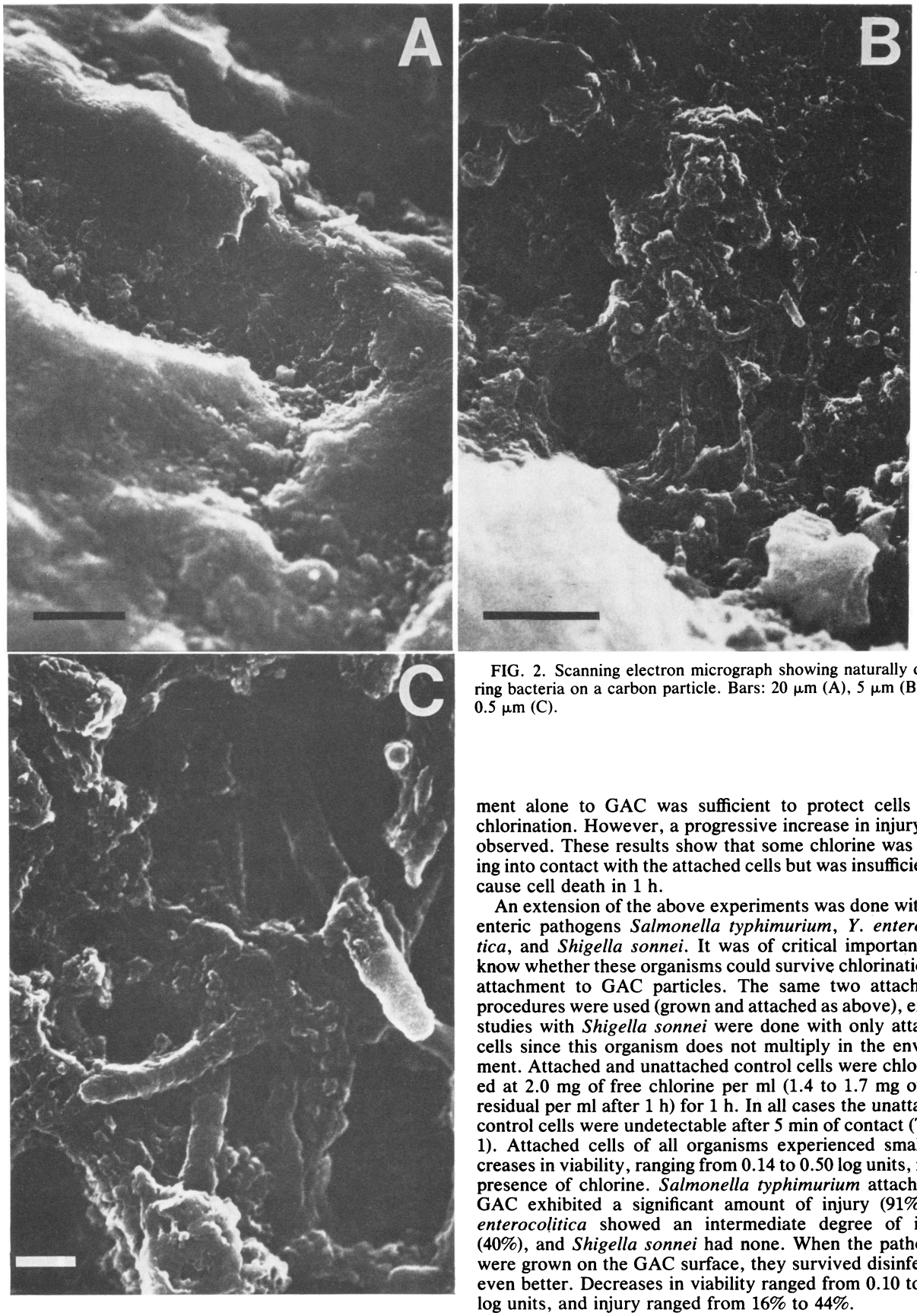

FIG. 2. Scanning electron micrograph showing naturally occurring bacteria on a carbon particle. Bars: 20 μm (A), 5 μm (B), and 0.5 μm (C).

ment alone to GAC was sufficient to protect cells from chlorination. However, a progressive increase in injury was observed. These results show that some chlorine was coming into contact with the attached cells but was insufficient to cause cell death in 1 h.

An extension of the above experiments was done with the enteric pathogens *Salmonella typhimurium*, *Y. enterocolitica*, and *Shigella sonnei*. It was of critical importance to know whether these organisms could survive chlorination by attachment to GAC particles. The same two attachment procedures were used (grown and attached as above), except studies with *Shigella sonnei* were done with only attached cells since this organism does not multiply in the environment. Attached and unattached control cells were chlorinated at 2.0 mg of free chlorine per ml (1.4 to 1.7 mg of free residual per ml after 1 h) for 1 h. In all cases the unattached control cells were undetectable after 5 min of contact (Table 1). Attached cells of all organisms experienced small decreases in viability, ranging from 0.14 to 0.50 log units, in the presence of chlorine. *Salmonella typhimurium* attached to GAC exhibited a significant amount of injury (91%). *Y. enterocolitica* showed an intermediate degree of injury (40%), and *Shigella sonnei* had none. When the pathogens were grown on the GAC surface, they survived disinfection even better. Decreases in viability ranged from 0.10 to 0.37 log units, and injury ranged from 16% to 44%.

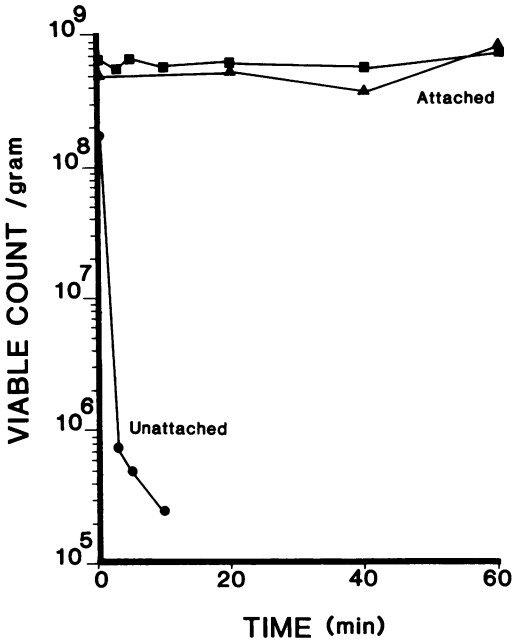

FIG. 3. Survival of *E. coli* grown on GAC exposed to 2.0 mg of chlorine per liter for 1 h (free chlorine residual after 1 h was 1.4 to 1.6 mg/liter). Symbols: ■, nonselective TLY agar; ▲, selective TLY-D agar.

DISCUSSION

Activated carbon particles can be released from GAC filter beds and can enter potable drinking water supplies (Abstr. Annu. Meet. Am. Soc. Microbiol. 1984. N33, p. 184). These particles are readily colonized by bacteria which may grow on the nutrient-rich, activated surface (3, 24). Data presented in this report show that these attached organisms are recalcitrant to the action of chlorine. Heterotrophic plate count bacteria, coliforms, and pathogens are all able to survive high disinfectant doses for long contact periods without significant decreases in viability.

Previous research has demonstrated that suspended particulates have a deleterious impact on the quality of drinking water (6, 7, 10, 12, 13, 18, 22). Water that was experimentally contaminated with feces containing infectious hepatitis virus and then chlorinated produced illnesses in volunteers, although a similar water sample that was treated with coagulation, filtration, and chlorination produced no morbidity (18). In this connection, LeChevallier et al. (13) showed at least a 90% reduction in disinfection efficiency when the turbidity of drinking water increased from 1 to 10 nephelometric turbidity units. Coliforms have also been associated with particulates such as crustaceans, nematodes, iron rust, and plankton inside distribution systems (6, 7, 22).

Suspended carbon particles can also carry nutrients to support microbial growth within distribution systems. Schalekamp (20) reported that microorganisms from chlorinated

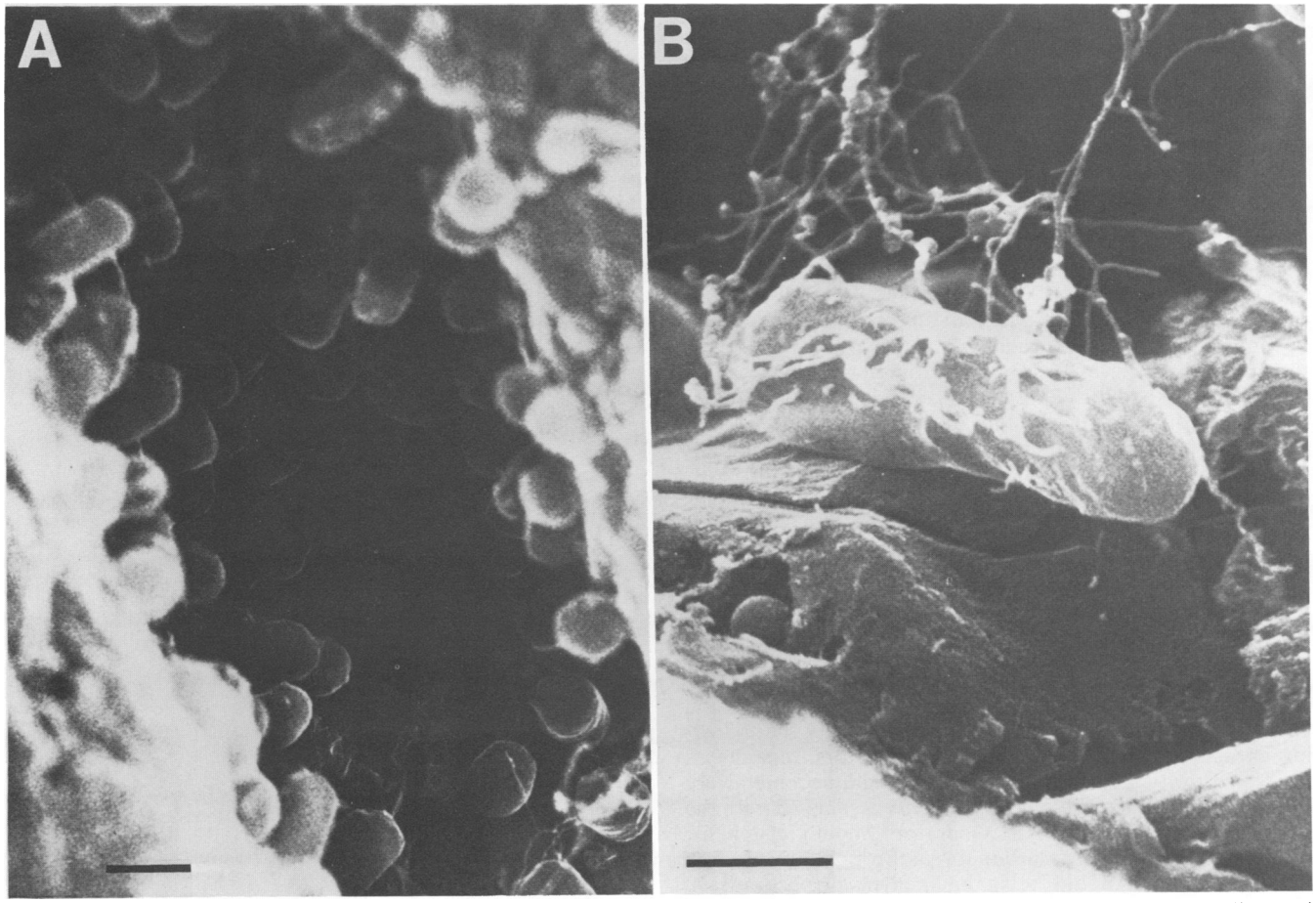

FIG. 4. Scanning electron micrograph of *E. coli* grown on GAC. (A) Dense cell growth in a crevice on a carbon particle; (B) cell coated with dehydrated extracellular polymer. Bars, 0.5 μm.

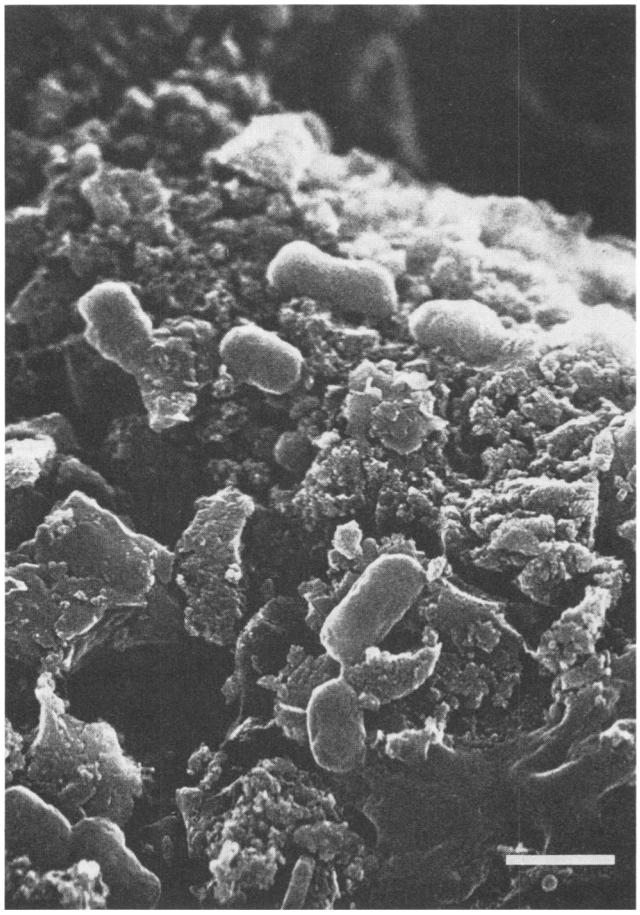

FIG. 5. Scanning electron micrograph of washed *E. coli* attached to virgin GAC. No observable extracellular polymer was produced. Bar, 1.0 μm.

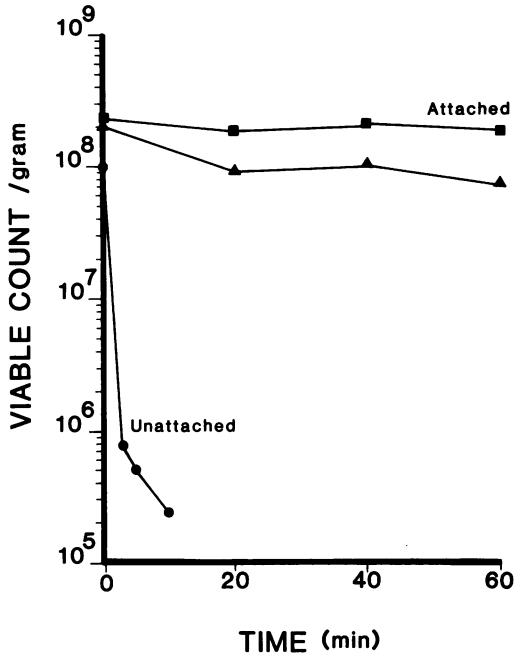

FIG. 6. Survival of washed *E. coli* attached to GAC exposed to 2.0 mg of chlorine per liter for 1 h (free chlorine residual after 1 h was 1.4 to 1.7 mg/liter). Symbols: ■, nonselective TLY agar; ▲, selective TLY-D agar.

GAC effluents regrew to levels of 10^3 to 10^4 CFU/ml in 3 days at 20°C in drinking water. Parsons et al. (Proc. AWWA Technol. Conf., Miami Beach, Fla., abstr. no. 3B-3, 1980) supports this observation, reporting that heterotrophic plate counts in finished drinking water, after carbon filtration, increased from 0 to 10^6 CFU/ml in 3 days.

Results from this report demonstrate that pathogenic microorganisms attached to GAC particles were highly resistant to chlorination. Preliminary work from ongoing studies identified *Salmonella* sp. on a GAC filter and enterotoxigenic (heat-stable) *E. coli* on carbon particles in GAC-treated drinking water. It must be emphasized, however, that these organisms were only occasionally isolated from drinking water or GAC and always in low numbers.

In summary, we have presented results showing that bacteria attached to carbon particles are not affected by chlorine disinfection. The hazard of attached bacteria in GAC-treated drinking water may be reduced by the following measures. (i) Treatment plant operators, engineers, health authorities, and administrators should become aware of the potential microbiological problems associated with the use of activated carbon. (ii) Operators should watch for turbidity increases and carbon breakthrough. (iii) A free chlorine residual should be maintained in all parts of the distribution system to prevent bacterial growth on carbon particles in drinking water. This is especially important in areas in which sediment will accumulate. (iv) An aggressive and continuous flushing program should be established to remove particles and sediment from the distribution system. Finally, further research is necessary to evaluate the extent and amount of carbon penetration through GAC filters. A more comprehensive study of the microbiological health effects associated with use of activated carbon is also required.

TABLE 1. Viability and injury of enteric pathogens on GAC with chlorine exposure

Species[a]	Time of chlorination (min)	Decrease in log viability[b]	% Injury[c]
Salmonella typhimurium			
Grown, 2 ppm of chlorine	60	0.37	44
Attached, 2 ppm of chlorine	60	0.50	9.1
Control	5	6	ND[d]
Yersinia enterocolitica			
Grown, 2 ppm of chlorine	60	0.10	16
Attached, 2 ppm of chlorine	60	0.40	40
Control	5	5	ND
Shigella sonnei			
Attached, 2 ppm of chlorine	60	0.14	0
Control	5	5	ND

[a] Data for cells grown on GAC are the average of two experiments; data for washed cells attached to GAC are the average of three experiments; control cells were washed once and suspended with no carbon.

[b] Log viability calculated by log TLY (nonselective) counts at 0 time minus log TLY counts at 60 min.

[c] Log injury calculated by log TLY-D (selective) counts at 0 time minus log TLY-D counts at 60 min.

[d] ND, Not determined.

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