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Microbial Resistance to Disinfectants: Mechanisms and Significance

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Drinking water disinfection provides the final barrier to transmission of a wide variety of potentially waterborne infectious agents including pathogenic bacteria, viruses, and protozoa. These agents differ greatly in their innate resistance to inactivation by disinfectants, ranging from extremely sensitive bacteria to highly resistant protozoan cysts. The close similarity between microorganism inactivation rates and the kinetics of chemical reactions has long been recognized. Ideally, under carefully controlled conditions, microorganism inactivation rates simulate first-order chemical reaction rates, making it possible to predict the effectiveness of disinfection under specific conditions. In practice, changes in relative resistance and deviations from first-order kinetics are caused by a number of factors, including microbial growth conditions, aggregation, and association with particulate materials. The net effect of all these factors is a reduction in the effectiveness and predictability of disinfection processes. To ensure effective pathogen control, disinfectant concentrations and contact times greater than experimentally determined values may be required. Of the factors causing enhanced disinfection resistance, protection by association with particulate matter is the most significant. Therefore, removal of particulate matter is an important step in increasing the effectiveness of disinfection processes.

Introduction

The primary purpose of the disinfection process in drinking water treatment is the control of waterborne diseases through inactivation of any pathogenic microorganisms present in the water. The high degree of success of this process used alone or in combination with other treatment processes is confirmed by the infrequent occurrence of waterborne disease outbreaks in modern times.

Interestingly, the disinfectant concentrations and contact times used by different water utilities vary widely. There are no Federal requirements regarding these parameters, but several states and advisory groups provide minimum requirements or recommendations. For instance, Washington State regulations require a free-chlorine residual of 0.2 mg/L after 30 min contact. Other recommended standards (*1*) call for 0.2 to 0.5 mg/L free residual chlorine with 30 min contact for groundwaters or 120 min contact for surface waters. For chloramines, 1 to 2 mg/L with 120 and 180 min contact time for groundwaters and surface waters, respectively, is recommended. Regulations and recommendations such as these have been developed and formalized over a period of years and are based in part on disinfection data and in part on practices that seemed prudent and that have consistently produced finished water that would meet coliform indicator standards.

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General Characteristics of Waterborne Pathogens

The usual waterborne pathogens comprise a diverse group of microorganisms and include bacterial, viral, and protozoan species. Some general physical and chemical characteristics of the pathogens are shown in Table 1. They range in size over nearly three orders of magnitude and vary greatly in the nature of their surface characteristics. These factors as well as their mode of reproduction and life cycles can have important effects on their survival in the environment and resistance to disinfectants. The viruses are extremely small and are strict parasites, relying on specific living host cells for their replication. The bacteria are more than 10-fold larger than viruses in diameter and are capable of reproducing independent of living hosts. The protozoa are approximately 10-fold larger than the bacteria. They have a complex life cycle, requiring a living host for completion of a portion of the life cycle, and exist outside the host in an inactive, resistant cyst form. The viruses have no metabolic processes nor cell membranes or other structures, whereas the bacteria and protozoa have these features along with the complex enzyme systems that are typical of cellular organisms.

Disinfection as a Kinetic Process

Inactivation of microorganisms by chemical disinfectants can be considered to have the characteristics of a first-order chemical reaction in which the slope of the

Table 1. Some characteristics of waterborne pathogens.

Type of pathogen	Shape	Size, μm	Specific examples	Nature of surface
Enteric viruses	Spherical	0.02–0.07	Hepatitis A virus Norwalk Agent Gastroenteritis virus(es)	Protein
Enteric bacteria	Cylindrical	0.3–0.5 × 2–4	Salmonella Shigella Escherichia Campylobacter	Lipopolysaccharide lipoprotein
Enteric protozoa (cyst form)	Ovoid	5–8 × 10–20	Giardia Entamoeba	Mucopolysaccharide?

Table 2. Inactivation of various microorganisms by free chlorine at 5°C, pH 6.0.[a]

Microorganism	Chlorine concn, mg/L	Inactivation time, min[b]	*Ct*
E. coli	0.1	0.4	0.04
Poliovirus 1	1.0	1.7	1.7
E. histolytica cysts	5.0	18	90[c]
G. lamblia cysts[d]	1.0	50	50
	2.0	40	80
	4	20	80
	8	9	72
G. lamblia cysts[d]	2.5	30	75
G. lamblia cysts[e]	2.5	100	250[c]
G. muris cysts	2.5	100	250[c]

[a]Data of Hoff, Rice, and Schaefer (*4*).
[b]For 99% inactivation.
[c]Extrapolated data.
[d]Cysts from symptomatic carriers.
[e]Cysts from asymptomatic carriers.

inactivation curve is dependent on the disinfectant type, species, and concentration as well as the microorganism being inactivated and environmental conditions. Although actual data often show deviation from first-order kinetics, it is a useful concept for evaluating the comparative effectiveness of disinfectants or the comparative resistance of microorganisms. A number of different methods for expressing these comparisons have been proposed. Baumann and Ludwig (*2*) described a simple approach based on the disinfectant concentration and exposure time required to inactivate a certain proportion of a specific microbial population under specific conditions of temperature and pH.

Based on data available at that time in the literature, they prepared curves as shown in Figure 1 and employed the general equation:

$$C^n t = K$$

in which C is the disinfectant concentration (mg/L), t is the contact time required for a given percentage of inactivation, n is a positive constant indicating the slope of the inactivation curve for a given microorganism at a given C and t, and K is a constant for a specific microorganism at a given water temperature and pH.

Their analysis of these data indicated that most of the disinfection curves had a slope of -1. In this case, the general equation becomes $Ct = K$. Although this concept was used in 1962, it received little attention until reexamined and used in a comprehensive review of disinfection data by a National Research Council Committee (*3*).

The range of Ct values shown by different types of potentially waterborne pathogens for chlorine is shown in Table 2 (*4*). All these data represent free-chlorine residual at pH 6.0 and 5°C. The Ct values clearly show that different types of pathogens vary tremendously in their comparative resistance to chlorine, ranging over about four orders of magnitude. The data also show that within the protozoan group, Ct varies somewhat, but the differences are relatively minor compared to the differences between groups. Similar variability occurs among the enteric bacteria and viruses.

Aberrations from Predicted Inactivation Rates

Ideally, based on the use of the Ct value, one should be able to achieve any desired level of pathogen inactivation by varying the disinfectant concentration and/or exposure time needed for the particular microorganism and environmental conditions (pH, temperature). However, a number of factors may alter the slope of the inactivation curve or cause deviations from the expected first-order inactivation rates. The types of curves that may be seen are shown in Figure 2. Curves *A* and *B* show first-order inactivation at different rates. These curves would indicate inactivation of two homogeneous populations with differing inherent resistance to the disinfectant. Curve *C* shows a typical deviation from first-order kinetics, characterized by an initial, relatively linear inactivation rate with a gradual slowing and "tailing off" of the rate. Curves such as this are typical of nonhomogeneous populations with different inherent resistance or protection of a portion of the population by extraneous factors such as shielding through association with particles or clumps of the organism.

Typically, linear inactivation rates are usually maintained through at least two to three orders of magnitude of inactivation. It is mainly for this reason that the 99% inactivation level is usually used in calculating comparative Ct values. As shown in Table 2, different isolates

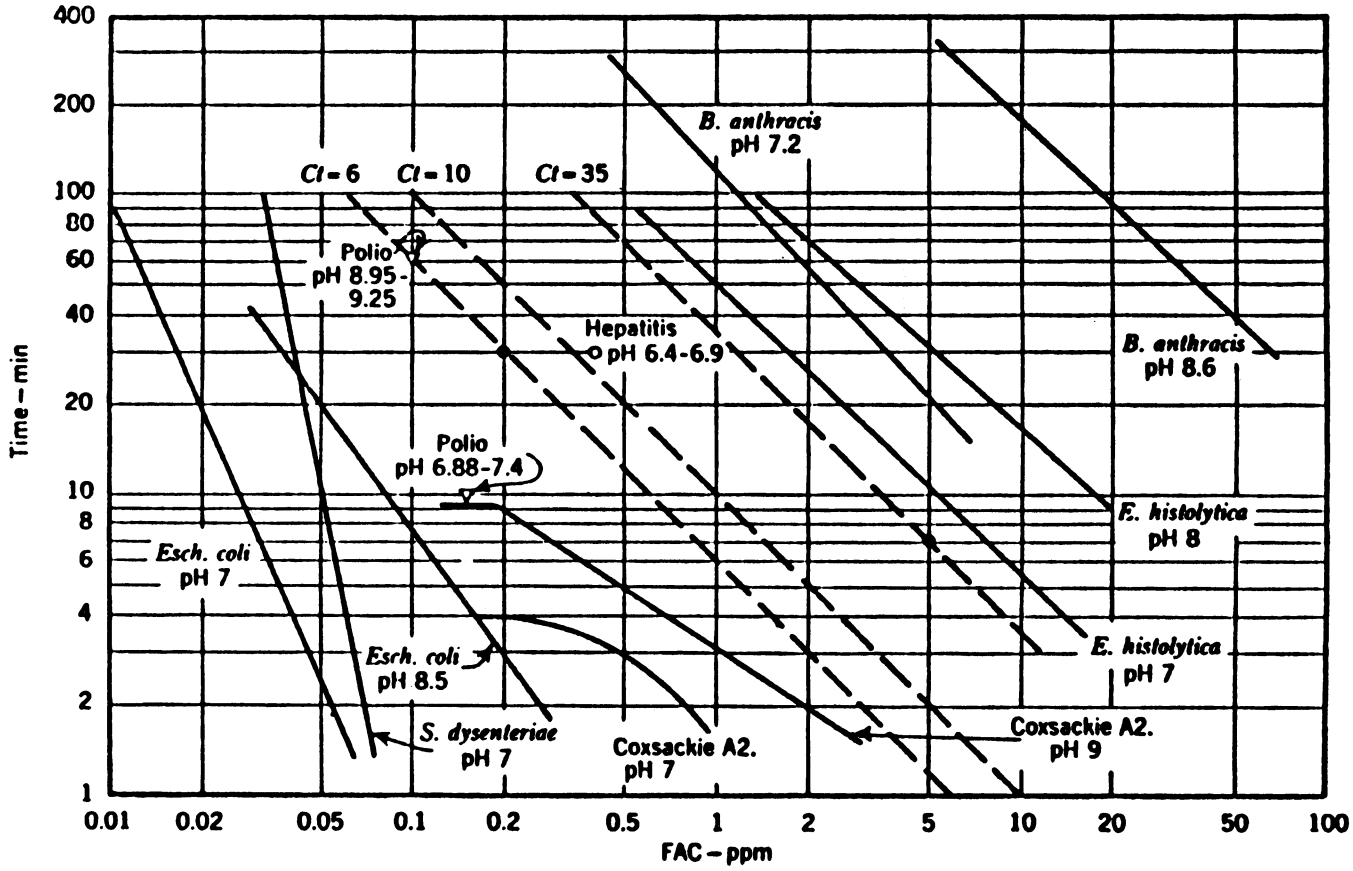

FIGURE 1. Inactivation of microorganisms by free chlorine at 20–29°C. From Baumann and Ludwig (*2*). Reproduced with permission.

of the same species (*G. lamblia*) may vary in their inherent resistance to inactivation. The reasons for these variations are largely unknown as are the reasons for the large differences in the relative resistance among pathogenic bacteria, viruses, and protozoan cysts.

Considerable research on the mechanisms by which chlorine and other oxidants inactivate microorganisms has been conducted, and a variety of mechanisms have been suggested. Chang (*5*) observed that greater uptake of chlorine and more rapid inactivation of *E. histolytica* cysts occurred at low pH levels, at which the chlorine was present in the undissociated hypochlorous acid form. Kulikovski et al. (*6*) and others have implicated damage to cell membranes and inhibition of biochemical activities associated with cell membranes as mechanisms of inactivation. Inhibition of specific enzymes and enzyme systems have also been suggested as inactivating mechanisms. Other studies have shown that chlorine physically damages nucleic acids, and in viruses, both nucleic acids (*7*) and surface proteins (*8*) have been proposed as the critical activity sites for halogen disinfectants. Because these disinfectants are powerful oxidizing agents, it is very likely that they affect many vital functions, making it very difficult to determine a specific site or function on which they exert their lethal effect.

Genetically Based Altered Resistance

The possible development of genetically based, increased resistance to disinfectants by natural selection has also been studied. An early study (*9*) indicated that *Escherichia coli* survivors of repeated exposures to chlorine showed enhanced resistance to chlorine. Other investigators (*10,11*) found no increase in resistance in bacterial cultures repeatedly exposed to chlorine and/or iodine. In a more recent investigation, Haas and Morrison (*12*) showed that *E. coli* survivors of low degrees of inactivation by chlorine did not show altered sensitivity. Survivors of high degrees of inactivation appeared to be more sensitive to chlorine. They speculated that the enhanced sensitivity of progeny of chlorine-exposed cells might be caused by chlorine-induced mutations that caused loss of some factor influential in preventing a lethal activity of chlorine such as the ability to repair nucleic acid damage or cell envelope damage. Bates et al. (*13*) reported that poliovirus type-1 subjected to repeated chlorine exposure cycles developed increased resistance to chlorine. *Ct* values (99% inactivation) estimated for their data (see Fig. 3) for virus subjected to 1, 5, and 10 chlorine exposure cycles are

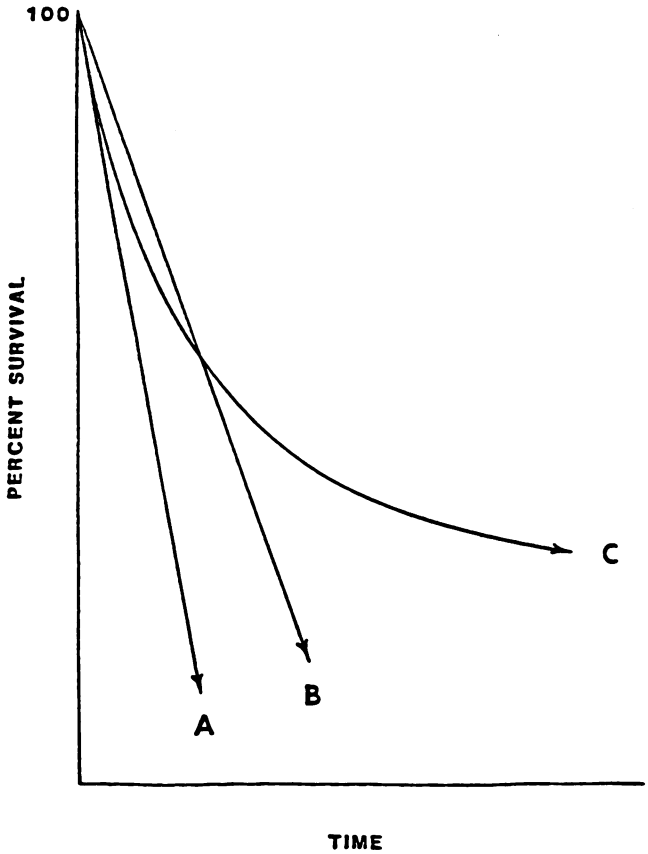

FIGURE 2. Effects of microorganism type and state on disinfection survival curves: (*A*) sensitive homogeneous populations; (*B*) more resistant homogeneous population; (*C*) heterogeneous population or partially protected by aggregation, etc.

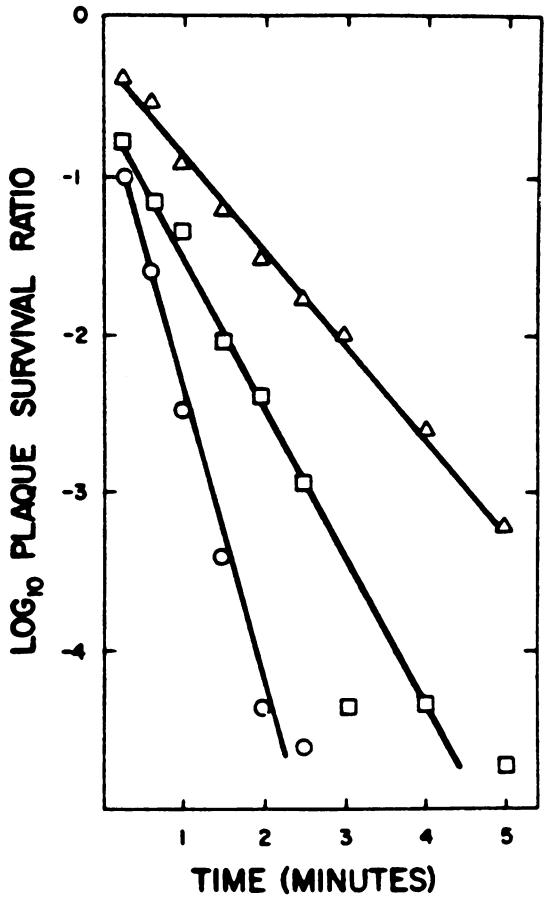

FIGURE 3. Effect of cyclic exposure to chlorine on poliovirus resistance: (○) cycle 1; (□) cycle 5; (△) cycle 10. From Bates et al. (*13*). Reprinted by permission.

0.54, 0.9, and 1.8, respectively, constituting about a threefold increase in *Ct* value after 10 exposure cycles. The increase in resistance did not proceed incrementally on a consistent basis. Viruses from some subsequent exposure cycles were less resistant than in previous cycles. Altered resistance to chlorine similar to that seen in the case of bacterial resistance to antibiotics has not been demonstrated. The halogen disinfectants appear to be general cytoplasmic poisons that affect many vital functions, and the development of genetically based resistance to such a variety of injurious actions seems unlikely.

Growth Condition Effects

There is increasing evidence that the disinfectant resistance of bacteria grown in laboratory cultures may differ greatly from that of the same species occuring in the environment. Favero and Drake (*14*) showed that *Pseudomonas alcaligenes*, a common bacterial contaminant in iodine-treated swimming pools, could grow well in swimming-pool water. Water-grown cultures were much more resistant to iodine that the same isolates grown in laboratory media. Carson et al. (*15*) showed that *Pseudomonas aeruginosa* grown in a natural water environment was much more resistant to inactivation by a variety of disinfectants, including chlorine dioxide, quaternary ammonium compounds, acetic acid, and glutaraldehyde, than the same *P. aeruginosa* isolate grown on conventional laboratory media. Subsequent studies have shown similar effects for *E. coli* (*16*), *Klebsiella pneumoniae* (*17*), *Yersinia enterocolitica* (*17*), and *Legionella pneumophila* (*18,19*). The studies using *P. alcaligenes* (*14*) and *P. aeruginosa* (*15*) and one of the studies involving *L. pneumophila* (*19*) were conducted with cultures grown using water as the medium. The data from these studies show essentially first-order inactivation rates with the slopes varying according to growth conditions as illustrated by Figure 4 (*19*). In this case, water-grown *L. pneumophila* required nearly 10 times as long for equivalent inactivation as agar-grown *L. pneumophila*. Other studies show that water-grown *L. pneumophila* subcultured on agar media immediately lost the enhanced resistance.

In several of the other studies (*16–18*), chemostat-grown cultures were used, with growth rates of the cultures controlled by limiting nutrients. These studies also showed differences in sensitivity to inactivation, but the inactivation curves were characterized by initial

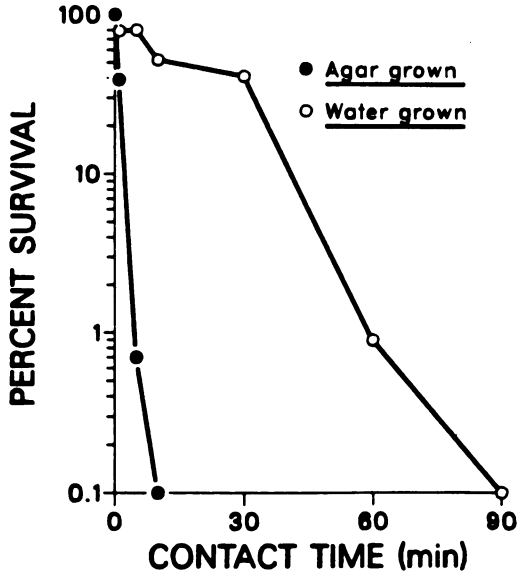

FIGURE 4. Effects of growth conditions on resistance of *L. pneumophila* to chlorine. From Kuchta et al. (*19*). Reprinted by permission.

rapid inactivation followed by "tailing off." The surviving fraction at the lower end of the curves varied according to growth conditions, with the highest level of survival occurring when nutrient levels and temperatures were lowest. The reasons for such changes in disinfectant resistance are not known. Changes in cell composition, altered membrane permeability, or formation of protective slime layers have been proposed as mechanisms, but definitive evidence is lacking. These studies indicate the effects of bacterial growth conditions on bacterial resistance to disinfectants and pose questions about the adequacy of disinfection data based on the use of laboratory cultures of certain pathogenic and indicator bacteria. However, most of the bacterial pathogens responsible for drinking-water-related disease outbreaks multiply in the intestinal tract and do not multiply in aquatic environments. Whether bacterial pathogens grown in the intestinal tract are more resistant to inactivation than when grown on laboratory media is not known. *Campylobacter jejuni* grown in mice appear to be as sensitive to chlorine as laboratory-grown cells (*20*).

Particle Association Effects

For disinfection to be effective, contact between the disinfectant and the microorganism must occur. With few exceptions (e.g., *L. pneumophila*), the major source of pathogens of potential health significance in drinking water is the feces of man or other animals. Thus, these pathogens are initially associated with particulate matter. The fate of these associations and the association of pathogens with other types of particles is influenced by many factors. Microorganism size is an important factor. Viruses, because of their small size, can be protected from disinfectant contact by much smaller particles than those which can protect bacteria or cysts. In addition, the surface charges involved in the sorption phenomenon exert more influence on viruses, because of their large surface-to-mass ratios, than on bacteria or cysts.

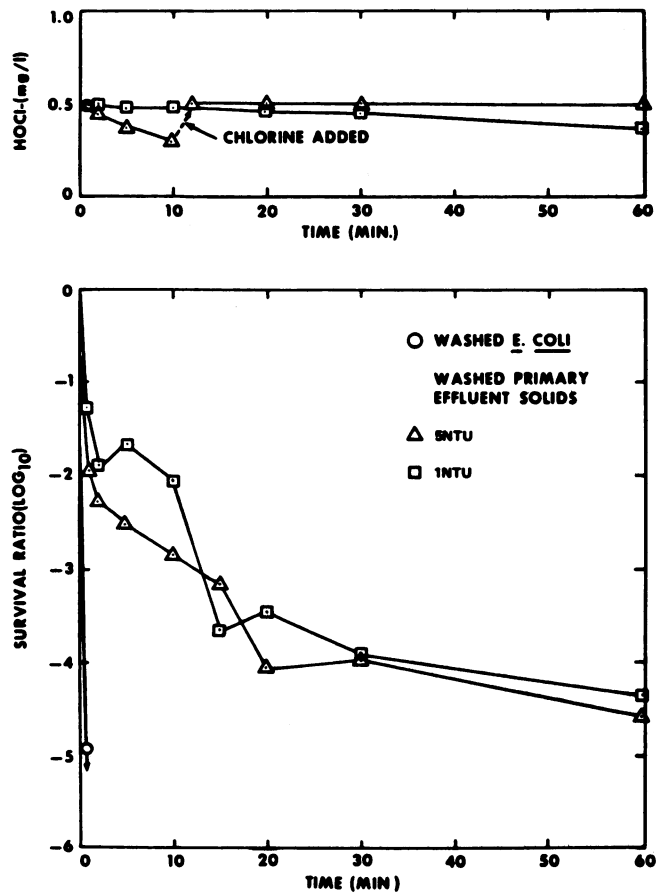

FIGURE 5. Inactivation of coliforms associated with primary effluent solids by chlorine (pH 6.0, 5.0°C). From Hoff (*22*).

Although the concept that certain particles may protect microorganisms from inactivation by disinfectants is logical and indeed provides the major basis for regulating drinking water turbidity, little direct evidence of such protection has been available until recently. In general, the results of these studies indicate that the effects of microorganism-particle association on disinfection efficiency are determined by the nature of the association. In the case of viruses and bacteria adsorbed on surfaces of particles such as clays or inorganic flocs, inactivation rates are unaffected or are affected only minimally (*21–25*).

In contrast, viruses associated with cell debris (simulating the conditions under which they are produced), feces, or wastewater effluent solids are substantially protected (*22,26,27*). The protective effects of wastewater effluent solids on coliform bacteria are shown in Figure 5 (*22*). Again, inactivation rates are initially very rapid, followed by a leveling off and survival of a small

fraction of the population after very long contact times. A more graphic illustration of the manner in which naturally occurring viral pathogens may be substantially protected is shown in Figure 6 (*28*). This electron micrograph shows large aggregates of membrane-associated rotavirus in human stool specimens. It is evident that the viruses in such complexes would be protected from inactivation by disinfectants. It is significant that the types of material that have been shown to offer protection are those materials which constitute the major sources of waterborne pathogens and would be associated with pathogens in contaminated waters.

Significance for Water Treatment

The overall effect of the factors that alter microbial resistance to inactivation by disinfectants is one of making drinking water disinfection processes less effective and less predictable than data derived from laboratory experiments would predict. To overcome these uncer-

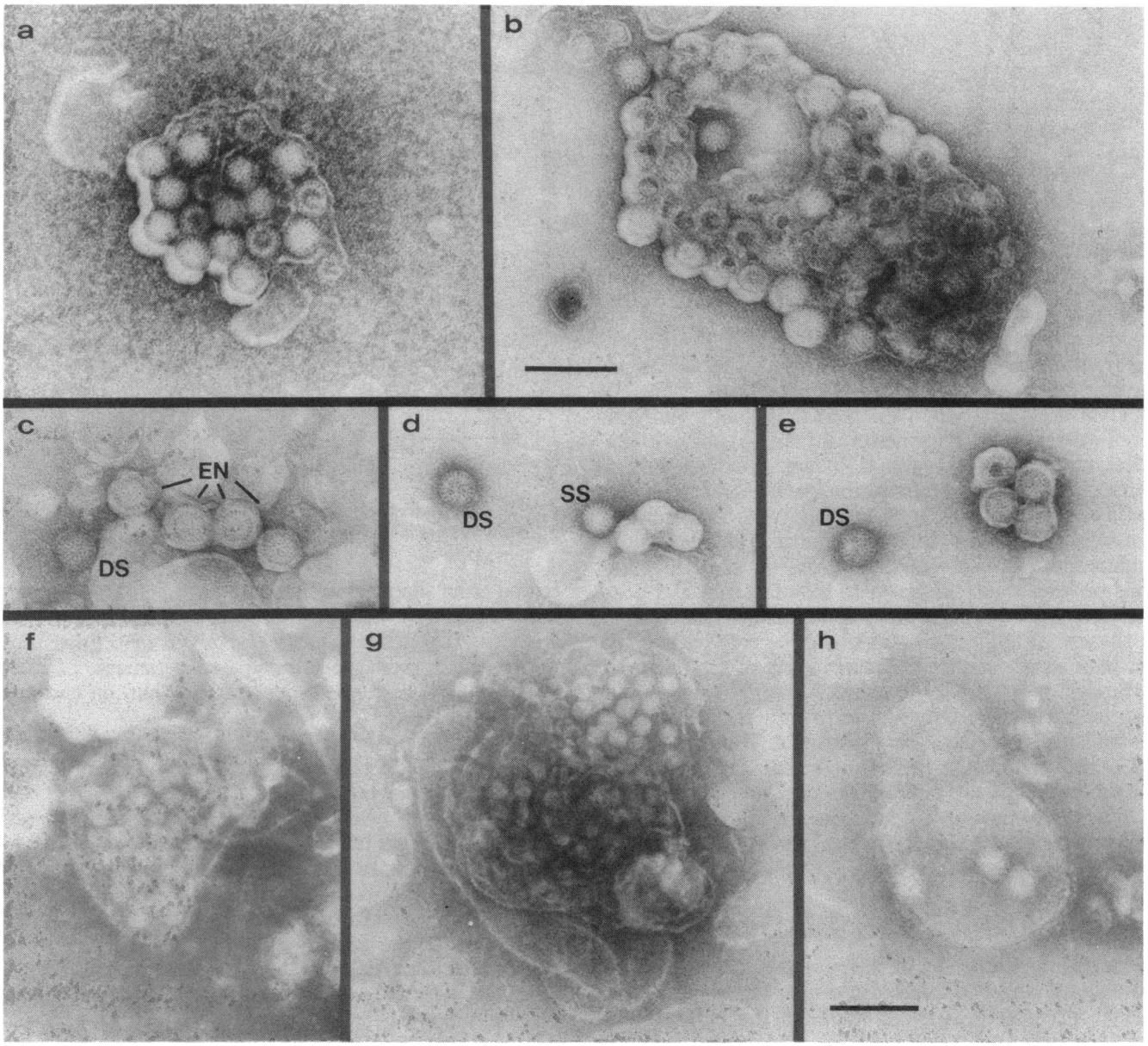

FIGURE 6. Membrane-associated virus particles in stool specimens. (*a,b*) viral-packet complexes of rotavirus in 1 specimen. (*c,d,e*) individual enveloped particles (EN), double-shelled particles (SS), and small groups of membrane-associated particles in the same specimen (DS). (*f,g*) parvoviruslike viral packets observed in another stool specimen. (*h*) three Norwalk virus particles associated with a fuzzy membraneous element in another stool specimen. Bar for panels *a* through *e*, 0.2 μm. Bar for panels *f* through *h*, 0.1 μm. From Williams (*28*). Reprinted by permission.

tainties and build in additional safety factors, higher disinfectant doses and longer contact times are often used. These practices increase chemical by-product formation and thereby increase the potential health problems related to halogenated organics.

The most significant enhanced resistance mechanism is that associated with the protective effects of particulate matter. It is the most significant factor for two reasons. First, because of the nature and sources of the particles that offer the most protection, pathogens are very likely to be associated with them. Second, particle association provides the highest degree of increased protection for the microorganisms studied. Fortunately, this mechanism is also the most amenable to control during water treatment. The particle removal processes used in water treatment (coagulation, flocculation, sedimentation filtration) when conducted properly are very effective in achieving high levels of particle removal, including removal of the pathogens themselves. This removal process, in turn, increases the reliability and predictability of the disinfection step. However, data from a few studies suggest that some viruses may exist in a physical state that allows them to survive sedimentation, filtration, and disinfection processes (*29,30*). Whether more vigorous control of conventional water treatment processes could eliminate these residual viruses or whether this degree of virus contamination poses a health hazard is yet to be determined.

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