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Inactivation of Human and Simian Rotaviruses by Chlorine Dioxide

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The inactivation of single-particle stocks of human (type 2, Wa) and simian (SA-11) rotaviruses by chlorine dioxide was investigated. Experiments were conducted at 4°C in a standard phosphate-carbonate buffer. Both virus types were rapidly inactivated, within 20 s under alkaline conditions, when chlorine dioxide concentrations ranging from 0.05 to 0.2 mg/liter were used. Similar reductions of 10^5-fold in infectivity required additional exposure time of 120 s at 0.2 mg/liter for Wa and at 0.5 mg/liter for SA-11, respectively, at pH 6.0. The inactivation of both virus types was moderate at neutral pH, and the sensitivities to chlorine dioxide were similar. The observed enhancement of virucidal efficiency with increasing pH was contrary to earlier findings with chlorine- and ozone-treated rotavirus particles, where efficiencies decreased with increasing alkalinity. Comparison of 99.9% virus inactivation times revealed ozone to be the most effective virucidal agent among these three disinfectants.

Human rotaviruses (HRV), members of the Reoviridae family of RNA viruses (11), are responsible for many of the reported cases of acute epidemic or endemic diarrhea affecting both children and adults (4, 12, 21, 23). The increasing number of reports associating rotaviruses with new clinical situations (5, 6, 8–10, 22) emphasizes the need to understand their epidemiology and transmission. Because these organisms may be disseminated through aquatic environments, it is important to examine the effectiveness of various modes of water disinfection on the inactivation of rotaviruses.

Traditionally, laboratory-based studies of water disinfectant efficacy have centered on the commonly used disinfectants, such as chlorine and ozone. More recent studies have addressed the inactivation potential of additional agents, including chlorine dioxide. This agent has been shown to be an effective bactericide (2, 14) and sporicide (13), as well as a potent virucide (1, 15). In addition, the use of chlorine dioxide as a water disinfectant does not result in the production of trihalomethanes (16), a problem associated with chlorine treatment of drinking water. Scarpino et al. (15) reported that chlorine dioxide inactivated poliovirus type 1 and enteroviruses more efficiently at pH 9.0 than at neutral or acidic levels. A similar pH effect was demonstrated in other experiments with poliovirus (1) and simian rotavirus SA-11 (3). To date, the only documented evidence for the inactivation of human rotavirus by chlorine dioxide was that reported by Harakeh and Butler (7). In these experiments, human rotavirus suspended in wastewater effluent was found to be somewhat less sensitive to treatment by chlorine, chlorine dioxide, ozone, and peracetic acid than the simian strain.

In the present study, the inactivation of purified, single-particle suspensions of simian (SA-11) and HRV by chlorine dioxide were compared over a range of disinfectant concentrations and pH levels. Resulting data were then compared with those from previous investigations of rotavirus inactivation by the more traditional agents, chlorine and ozone (19, 20).

MATERIALS AND METHODS

Simian rotavirus SA-11 obtained from Charles Gerba, University of Arizona, Tucson, and HRV type 2 (Wa), purchased from Biotech Research Laboratories, Rockville, Md., were used in all studies. Host cell cultures (MA-104) were purchased from Microbiological Associates, Walkersville, Md. Virus propagation, purification, and assay were carried out as previously described (19, 20). Chlorine dioxide stock solutions were prepared in a chlorine-demand-free phosphate-carbonate buffer according to the method of Benarde et al. (2), with fresh solutions (approximate concentration, 200 mg/liter) stored at 4°C in air-tight dark glass bottles for periods of up to 2 weeks. The concentration of chlorine dioxide was determined by the method of Roller et al. (14), with A_{357} measured in a dual-beamed spectrophotometer (model Acta III; Beckman Instruments, Inc., Fullerton, Calif.).

Prior to each experiment, chlorine dioxide stock solution was diluted to the desired concentration with chlorine-demand-free buffer. One-hundred-milliliter volumes of chlorine dioxide containing buffer were then inoculated with 1 ml of dialyzed single-particle virus stock (~10^7 PFU/ml) and gently mixed on a magnetic stirrer. Samples (10 ml each) were collected at intervals and placed in test tubes containing 0.1 ml of 0.5 M sodium thiosulfate to terminate the reaction. All samples were then treated with 0.5 ml of chloroform for 10 min to eliminate microbial contamination, diluted in Tris-buffered saline, and assayed as previously described (19).

To verify that host cells were both virus susceptible and contaminant free, positive and negative rotavirus controls were included in each experiment. Each experiment was repeated several times (usually two to three) to assure consistency of the results. Data were statistically analyzed according to the methods described by Sokal and Rohlf (17) and Steel and Torrie (18). Statistical analyses and graphics were performed on a Macintosh SE computer with preprogrammed statistical software.

RESULTS

The concentrations of chlorine dioxide working stocks maintained at 4°C were stable for 10 min. The dissipation of

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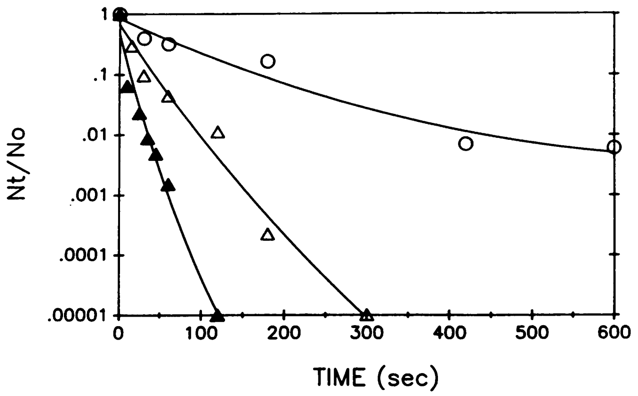

FIG. 1. Inactivation of SA-11 by chlorine dioxide at pH 6.0. The chlorine dioxide concentrations in milligrams per liter were as follows: ○, 0.05; △, 0.3; and ▲, 0.5. Nt/No, Number of virus PFU at a given time/number of virus PFU at zero time.

chlorine dioxide during the course of each experiment (maximum 10 min) averaged 0.02 mg/liter. All chlorine dioxide residuals reported below represent those measured immediately prior to each experiment. Datum points on each curve are median values of several separate experimental runs.

The results of SA-11 inactivation studies conducted at pHs 6 and 7 are presented in Fig. 1 and 2. At these levels, SA-11 was fairly tolerant to the treatment of chlorine dioxide at concentrations as high as 0.17 mg/liter. When the disinfectant concentration was increased to 0.5 mg/liter, 10^5-fold virus reduction times were reduced to 2 min (pH 6) and 30 s (pH 7). The inactivation of HRV by chlorine dioxide was moderate at acid and neutral pHs, with a disinfectant concentration of 0.2 mg/liter required to effect a 10^5-fold reduction infectivity within 2 min at pH 6 (Fig. 3) and within 3 min at pH 7 (Fig. 4). Inactivation of both virus types was similar at neutral pH, while HRV appeared to be somewhat more sensitive at pH 6.0.

Both human and simian rotaviruses were rapidly inactivated at pH 8 (Fig. 5), with residuals of 0.2 mg/liter causing complete inactivation (reduction of 10^5 PFU) within 15 s. This enhancement of disinfection with increasing pH was contrary to the phenomena previously described for chlorine and ozone (19, 20).

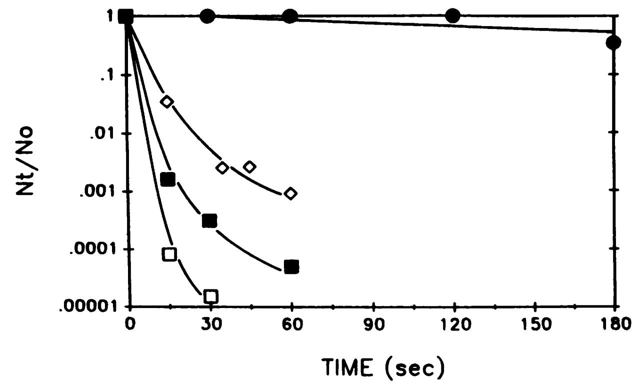

FIG. 2. Inactivation of SA-11 by chlorine dioxide at pH 7.0. The concentrations of chlorine dioxide in milligrams per liter were as follows: ●, 0.11; ◇, 0.17; ■, 0.40; and □, 0.6. Nt/No, Number of virus PFU at given time/number of virus PFU at zero time.

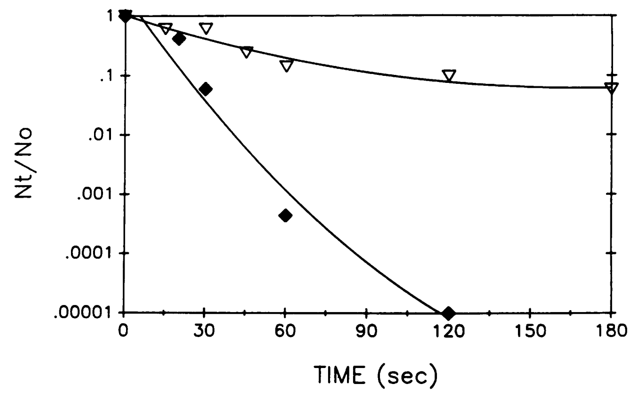

FIG. 3. Inactivation of HRV by chlorine dioxide at pH 6.0. The chlorine dioxide concentrations in milligrams per liter were as follows: ▽, 0.15; and ◆, 0.20. Nt/No, Number of virus PFU at a given time/number of virus PFU at zero time.

Data from the present study were compared with those from previous studies of chlorine and ozone-induced rotavirus inactivation in which identical experimental conditions were used (19, 20). Comparative data are presented in Table 1. Three-log (99.9%) virus inactivation times were derived by extending a line parallel to the *x* axis (time) from the *y* axis (Nt/No, number of viral PFU at a given time/that number at zero time) to its point of intersection with each inactivation curve and then locating its corresponding point on the *x* axis.

Direct comparison of 99.9% inactivation times at specific ozone and chlorine versus chlorine dioxide concentrations was complicated by the dilution-induced variability in the preparation of chlorine dioxide working stocks. In spite of this, several general trends were evident. While chlorine appeared to be somewhat more effective against SA-11 at pH 7.0, ozone was the most potent virucide overall. The effectiveness of chlorine dioxide was comparatively poor at acid and neutral pHs. Under alkaline conditions, however, its virucidal efficiency was enhanced to a level comparable to that of ozone.

DISCUSSION

The efficiency of virus inactivation by chlorine dioxide has been the subject of several laboratory investigations (1, 3, 7,

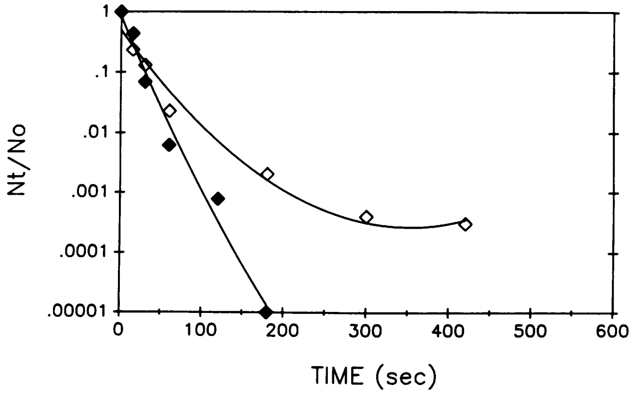

FIG. 4. Inactivation of HRV by chlorine dioxide at pH 7.0. The concentrations of chlorine dioxide in milligrams per liter were as follows: ◇, 0.1; and ◆, 0.2. Nt/No, Number of virus PFU at a given time/number of virus PFU at zero time.

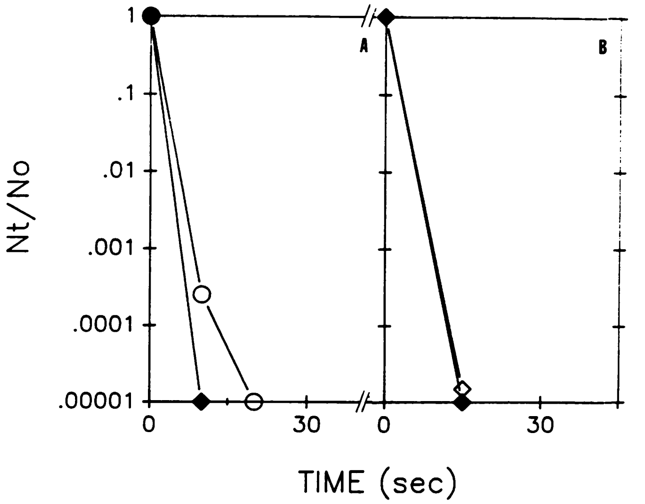

FIG. 5. (A) Inactivation of SA-11 by chlorine dioxide at pH 8.0. (B) Inactivation of HRV by chlorine dioxide at pH 8.0. The chlorine dioxide concentrations in milligrams per liter were as follows: ○, 0.05; ◆, 0.20; and ◇, 0.10. Nt/No, Number of virus PFU at a given time/number of virus PFU at zero time.

15). To date, none have used purified virus preparations and only one (7) addressed HRV inactivation.

In the present study simian rotavirus and HRV types were exposed as single particles to various chlorine dioxide concentrations and pH levels at 4°C in a chlorine-demand-free buffer system. Both virus types were most efficiently inactivated at pH 8, with 0.2 mg/liter residuals reducing 10^5-fold infectivity within 15 s. Similar alkaline enhancement of chlorine dioxide disinfection was previously reported for poliovirus (1), enteroviruses (15), and SA-11 rotavirus (3). Disinfection effectiveness decreased dramatically when the pH was lowered to 6.0. Although some differences were noted in the relative sensitivities of the test viruses to chlorine dioxide challenge, most notably at pH 6.0 where more than twice as much disinfectant was required to achieve a 10^5-fold reduction in SA-11 infectivity within 120 s, these differences were not considered to be significant within the context of the entire study.

Comparison of 99.9% virus inactivation times from the present study with those from recent studies with chlorine and ozone revealed chlorine dioxide to be the least efficient virucide at an acid or neutral pH. At an alkaline pH, however, chlorine dioxide-induced inactivation was superior to that of chlorine. Within the confines of this comparison of three disinfectants in the inactivation of rotaviruses, where identical experimental procedures were used, ozone appeared to be the most effective virucidal agent.

TABLE 1. Approximate times for 99.9% inactivation of SA-11 and HRV by chlorine, ozone, and chlorine dioxide at 4°C

Virus	pH	Disinfectant concn (mg/liter)	99.9% Inactivation time (seconds)		
			Chlorine[a]	Ozone[b]	Chlorine dioxide
SA-11	6.0	0.05			>600[c]
		0.17	20	8	
		0.25	6[d]	6	
		0.30			160
		0.50			60
	7.0	0.10	21	32	>180[c]
		0.20	6	9	60
		0.40			22
		0.60			12
	8.0	0.05			10
		0.10	28	6	
		0.20			6
HRV	6.0	0.10	46	6	
		0.15			>180[c]
		0.20	10	6	65
	7.0	0.10	60	6	210
		0.20	8	6	120
	8.0	0.10	39	6	12
		0.20	22	6	6

[a] From Vaughn et al. (20).

[b] From Vaughn et al. (19).

[c] Indicated time equals duration of respective experiments.

[d] A value of 6.0 s represents the shortest time which can be estimated from the inactivation curves. Since 10^5-fold inactivation was ≤10 s in these experiments, the actual 99.9% reduction was very likely <6 s.

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