BROMINE CHLORIDE:

AN ALTERNATIVE DISINFECTANT TO CHLORINE

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USE OF BROMINE CHLORIDE AS A VIRUCIDE IN TREATING WASTE WATERS

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ABSTRACT

Bromine chloride (BrCl) was evaluated as an alternative to chlorine as a disinfectant of water and waste water by comparing the efficiency of these two chemicals to inactivate type 1 poliovirus seeded in various aqueous solutions. In a nitrogen-free buffer at pH 6.0, the minimum concentration of BrCl required to effectively inactivate poliovirus (4-log reduction after 15 min at 25°C) was 0.15 mg/ℓ, whereas 0.3 mg/ℓ of chlorine was required to accomplish the same effect. The virus inactivating efficiency of BrCl was not interfered within the range of pH 6 to 10. Furthermore, the addition of various concentrations of glycine and NH_4Cl to the nitrogen-free buffer solutions more effectively interfered with the virus inactivating properties of chlorine than BrCl. To simulate waste water disinfection, 1 to 5 mg/ℓ of BrCl and chlorine were added to activated sludge treated sewage effluent seeded with poliovirus, mixed well, and titered after 15 min at 25°C. The results show that the inactivating effects of 1, 2, and 3 mg/ℓ dose of both chlorine and BrCl were equivalent and inefficient. However, at a dose of 5 mg/ℓ, BrCl inactivated 5 logs of virus, whereas chlorine inactivated only 2 logs of virus. These results indicate that BrCl should be seriously considered as a potential alternative to chlorine as a disinfectant.

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INTRODUCTION

Chlorine is used almost exclusively in the United States and many other countries to disinfect both drinking water and waste waters. However, the continued use of chlorine has been criticized for the following reasons: (1) human enteric viruses are more resistant to inactivation by chlorine than coliform bacteria and persist in chlorinated sewage effluents (Berg 1971; Chang 1970; Geldreich and Clarke 1971); (2) the disinfectant properties of chlorine are adversely affected by pH values greater than 7 and by nitrogenous compounds — conditions normally present in most natural and waste waters (White 1972); (3) chlorine forms very stable compounds which are toxic to aquatic life in waters receiving the discharge (Brungs 1973; Zillich 1972); and (4) chlorine may react with hydrocarbons found in natural waters to form potentially carcinogenic and mutagenic compounds, some of which have been detected in drinking water supplies in the U.S.A. (Bellar, Lichtenberg, and Kroner 1974; Dowty et al. 1975; Shih and Lederberg 1976). Thus, because of these problems, a more effective and less hazardous disinfectant than chlorine is currently being sought.

An alternative disinfectant which has not been adequately evaluated and has only recently been made available is bromine chloride (BrCl). Initial reports by Mills (1973, 1975) indicate that BrCl is an effective disinfectant which is economically competitive and less toxic than chlorine. Furthermore, it can be handled safely, and can be adapted to existing chlorination systems with only minor modifications (Dow Chemical Company 1972).

The objective of this investigation was to compare the virus disinfecting properties of BrCl and chlorine under controlled laboratory conditions and thereby determine whether BrCl should be considered as a viable alternative to chlorine as a water disinfectant. Poliovirus was selected as the representative sewage-borne enteric virus to be disinfected by these two chemicals since this virus is frequently present in waste waters receiving human wastes and is more resistant to chlorine disinfection than coliform bacteria.

MATERIALS AND METHODS

Cells, Media, and Virus

Monolayer cultures of BGM, a continuous line of African Green monkey kidney cells (Dahling, Berg, and Berman 1974) obtained from Gerald Berg (EPA, Cincinnati, Ohio), were grown in prescription bottles using Eagle's Basal Medium (EBM) with Hanks' or Earle's salts, and supplemented with 7% fetal calf serum (FCS) at 37°C in a CO_2 incubator. The purified type 1 (LSc2Ab) poliovirus used in all experiments, was grown in BGM cells for 48 hr at 37°C, concentrated by centrifugation, and was purified by treatment with Genetron (Allied Chemical) followed by banding in an isopycnic cesium chloride gradient. The banded virus was collected, dialyzed free of the cesium salt against phosphate buffered saline (PBS), and diluted for storage at 4°C in the same buffer.

Water and Glassware

The water used in all experiments was initially distilled using an American Sterilizer Company metal distiller, followed by deionization using a Crystalab demineralizer, and final redistillation in a Pyrex glass distillation apparatus. All glassware used in the experiments were made halogen-demand free by washing and soaking overnight in an acid cleaning solution, followed by thorough rinsing in tap and distilled water.

Chemicals, Reagents, and Sewage Effluent

Bromine chloride was supplied by Dow Chemical Company (Midland, MI.) in 12.7-kg (28-lb), or 2.04-atm (30-psi) cylinders provided with a liquid dip tube. A silver feedline, leading to a gas diffuser, was attached to the cylinder. By allowing the liquid BrCl to vaporize within the feedline, BrCl gas could be bubbled into solution. Saturated aqueous stock solutions prepared in this manner were stable upon storage in the dark at 4°C for several months. Chlorine solutions were prepared by dissolving calcium hypochlorite powder (Olin Matheson Chemical Company, New York, N.Y.) in distilled water. Both BrCl and chlorine were measured in nitrogen-free aqueous solutions as either HOBr or HOCl using a Wallace and Tiernan amperometric titrator. Values obtained for BrCl are multiplied by 1.62 to convert to

mg/ℓ BrCl. Nitrogen-free buffers used were 0.05*M* phosphate buffer (KH_2PO_4-K_2HPO_4), pH 5.8 to 7.7 and 0.05*M* borate buffer (H_3BO_3-NaOH-KCl), pH 8.1 to 10.0. Secondary treated (activated sludge) sewage effluent was obtained from the Mililani Sewage Treatment Plant, Oahu, Hawaii.

Experimental Design

For all virus inactivation studies, 50 mℓ of the test solution containing the halogens were prepared in 125-mℓ Erlenmeyer flasks and mixed at 25°C using a gyratory shaking waterbath (New Brunswick Scientific Company) and kept in a darkened room. While mixing, these solutions were inoculated with 1 mℓ of poliovirus (10^5 to 10^7 PFU/mℓ) and the mixture allowed to react for various periods of time not to exceed 15 min, as this represented the usual time under practical disinfection conditions. After the desired reaction time, 0.5-mℓ aliquots of the mixture were removed and immediately mixed into 4.5 mℓ of a cold pH 7 phosphate buffer containing 10 mg/ℓ of sodium thiosulfate ($Na_2S_2O_3$) to effectively stop the further halogen reaction. All samples were then assayed for virus by the plaque titration method. Inactivation was measured by determining the log V/V_o, where V is the titer of the sample after treatment and V_o the titer of the sample before treatment.

RESULTS

Effective Concentration of BrCl versus Chlorine

The virus inactivating effectiveness of BrCl (as HOBr) was initially compared against that of chlorine (as HOCl) under conditions known to be favorable to chlorine (pH 6, 25°C, and in nitrogen-free buffer). To determine the minimum concentration of BrCl versus chlorine required to effectively inactivate poliovirus, 50 mℓ of various concentrations of BrCl and chlorine were prepared in phosphate buffer at pH 6. To each of these solutions were added 1 mℓ of poliovirus (10^5 to 10^6 PFU/mℓ) followed by rapid mixing for 15 min in the dark, at 25°C. All samples were then immediately assayed for virus. The results in Figure 1 show that although 1 log of virus inactivation occurred when 0.1 mg/ℓ of BrCl was used, the minimum concentration of BrCl required for marked inactivation of poliovirus (>4 logs) was 0.15 mg/ℓ. In contrast, under similar conditions 0.3 mg/ℓ of chlorine was

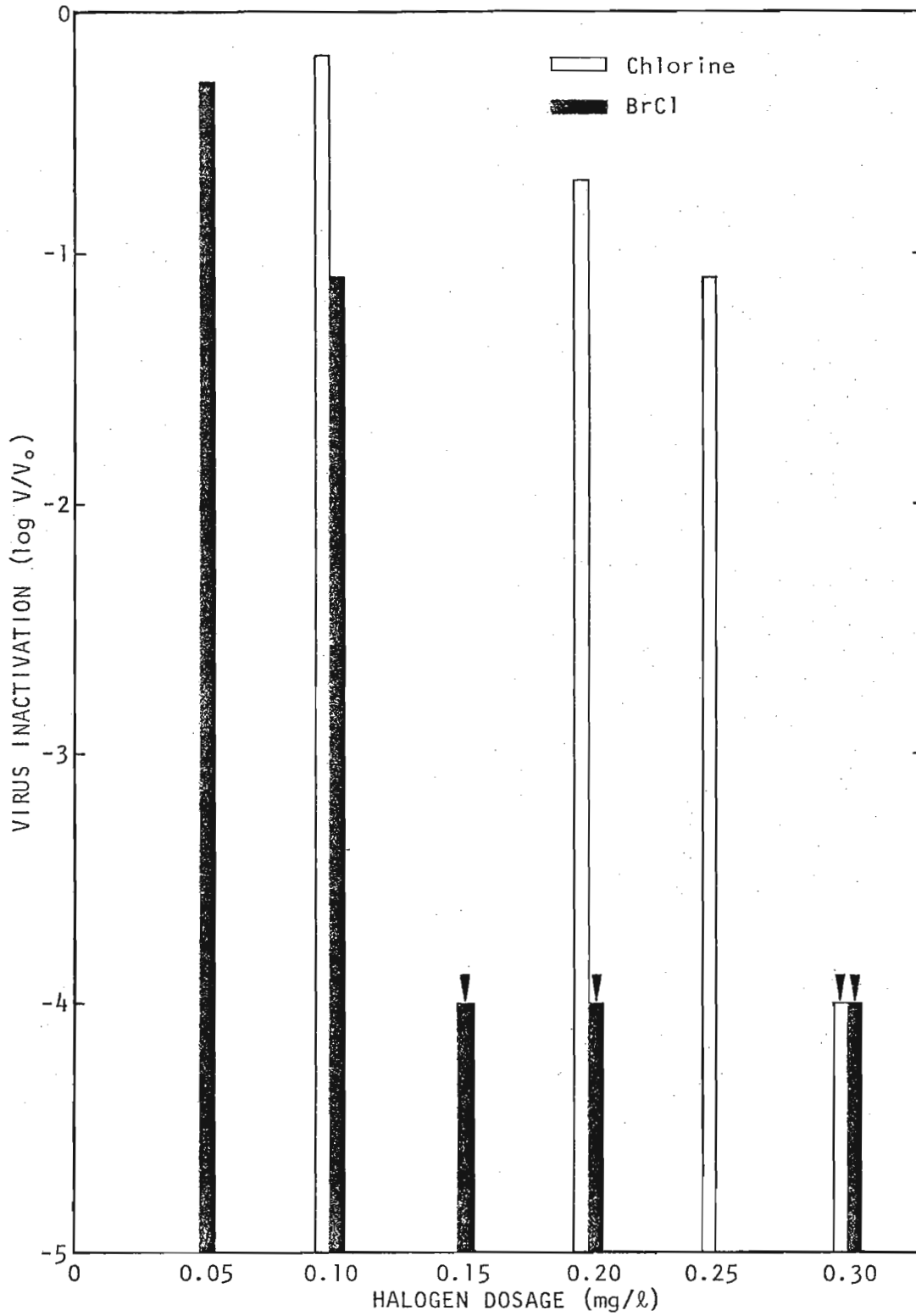

FIGURE 1. EFFECT OF CONCENTRATION OF BrCl VS. CHLORINE ON POLIOVIRUS INACTIVATION

required before an equivalent amount of poliovirus was inactivated. Thus, the minimum concentration of BrCl (0.15 mg/ℓ) required to effectively inactivate poliovirus under these conditions is about one-half that required for chlorine.

Stability of BrCl

It was initially observed that while the stock concentrations of BrCl (100 mg/ℓ) were stable and could be stored for several months when kept in the dark at 4°C, the dilute solutions of BrCl (0.15 mg/ℓ) were relatively unstable. To determine the stability of dilute concentrations of BrCl, 0.15 mg/ℓ samples of BrCl in nitrogen-free buffer (pH 7) were freshly prepared from the stock solutions. After various periods (2 to 45 min) of mixing at 25°C in the dark, poliovirus was added to these solutions, followed by vigorous mixing for 15 min at 25°C in the dark, and the samples were then immediately assayed for virus. The results in Figure 2 show that 0.15 mg/ℓ BrCl was stable for at least 15 min after the initial preparation, but rapidly lost much of its effectiveness to inactivate virus after 30 min of mixing in the dark. Therefore, in all subsequent experiments BrCl solutions were used 4 min after they were prepared from the stock solution.

Effect of pH

A major limitation of chlorine is that its disinfecting property is rapidly reduced as the pH of the environment increases above pH 7. To determine the effect of pH on the virus inactivating property of BrCl, 50-mℓ samples of 0.15 mg/ℓ of BrCl were made in nitrogen-free phosphate buffer at pH 6, 7.5, 8, 9, and 10. One mℓ of poliovirus (10^7 PFU/mℓ) was then added to each of these solutions, followed by vigorous mixing for 15 min at 25°C in the dark, and the samples were then taken and immediately assayed for virus. The pH values of these solutions were redetermined and found to remain unchanged under these conditions. The results in Figure 3 show that although some interference was observed at pH 8, 9, and 10, 0.15 mg/ℓ BrCl effectively inactivated poliovirus within the pH range of 6 to 10.

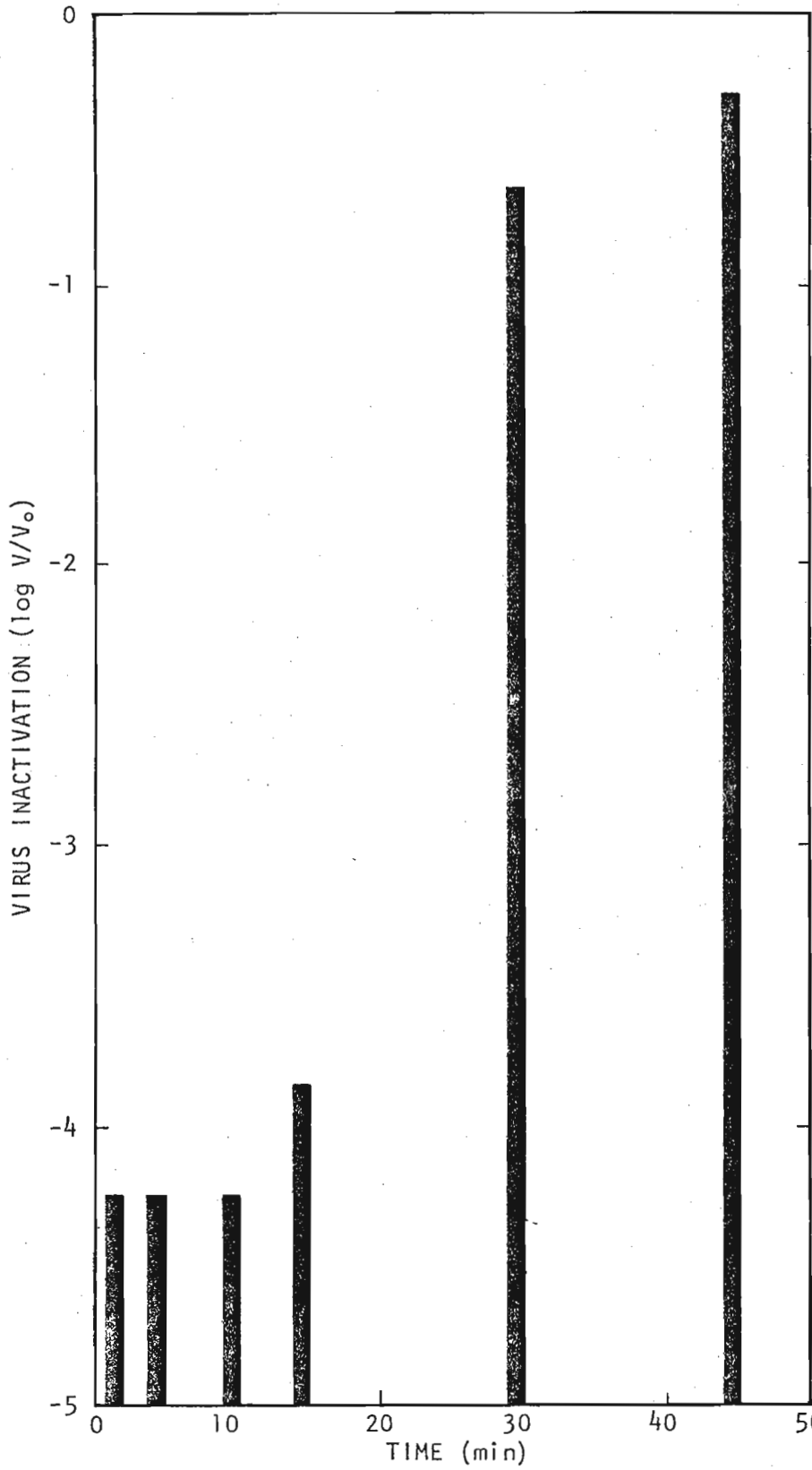

FIGURE 2. STABILITY OF MINIMAL EFFECTIVE CONCENTRATION OF BrCl

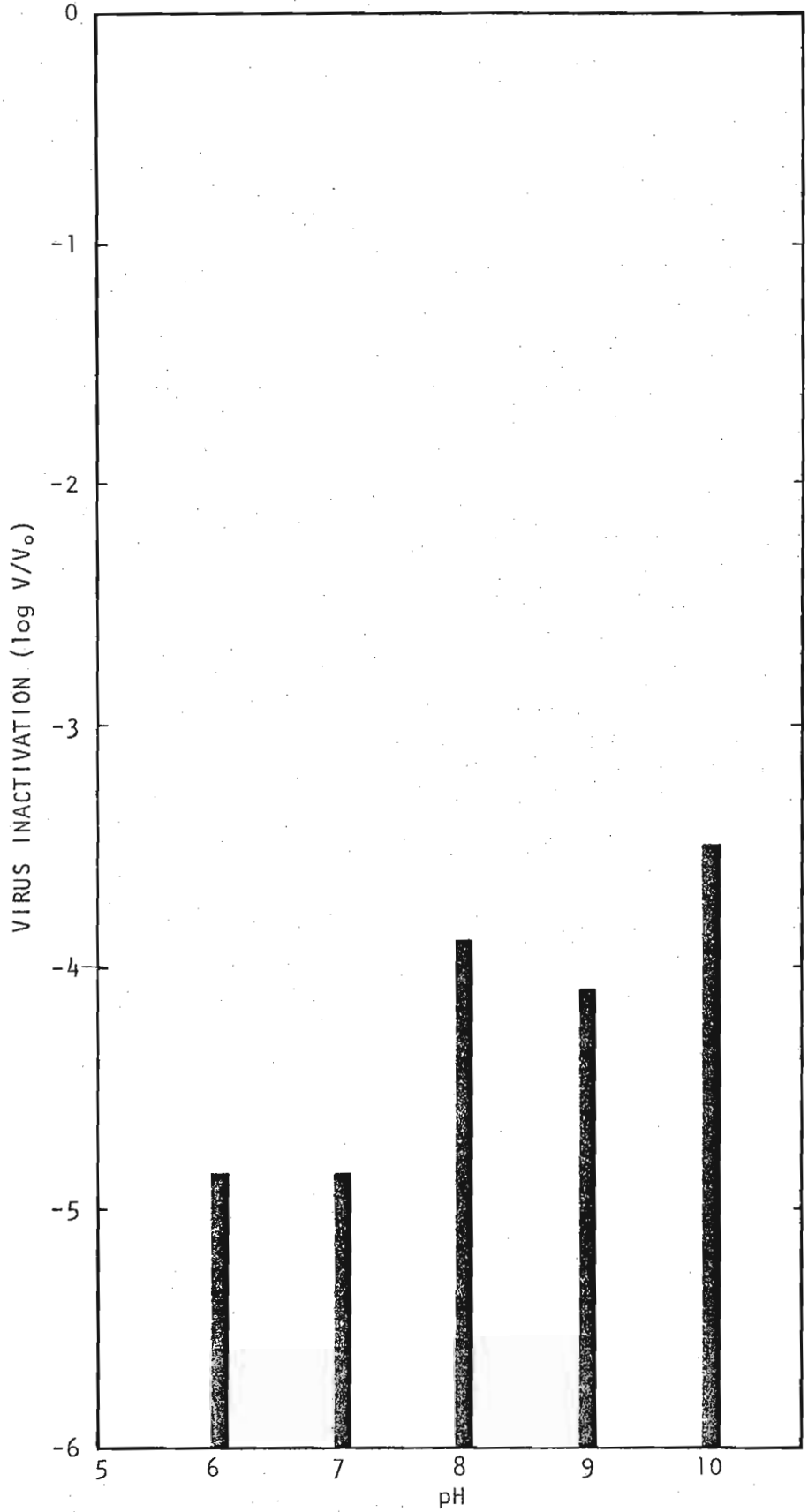

FIGURE 3. EFFECT OF pH ON POLIOVIRUS INACTIVATION BY 0.15 mg/ℓ BrCl IN A NITROGEN-FREE BUFFER

Kinetics of Inactivation of Poliovirus

Although the virus inactivation efficacy of BrCl was shown to be concentration dependent (Fig. 1), the kinetics and the minimum time required for virus inactivation were not determined. To accomplish this, 1 mℓ of poliovirus (10^5 PFU/mℓ) was added to 50-mℓ solutions of 0.075, 0.15, and 0.30 mg/ℓ of BrCl in nitrogen-free phosphate buffer at pH 7.5 and mixed in the dark at 25°C. At various time periods, 0.5-mℓ aliquots were removed, immediately diluted in phosphate buffer (4.5 mℓ, pH 7.5) containing 10 mg/ℓ sodium thiosulfate, and assayed for virus. The results in Figure 4 show that the inactivation of poliovirus by BrCl at the lower dosages (0.075 mg/ℓ and 0.15 mg/ℓ) was characterized by a biphasic curve—composed of an initial linear and rapid rate of inactivation followed by another linear but slower rate of inactivation representing a residual fraction of viable virus. As the dosage concentration of BrCl was increased (1.0 mg/ℓ), the rate of virus inactivation increased sharply and the size of the residual fraction of viable virus markedly decreased. It should be noted that the initial rate of virus inactivation was essentially completed within 2 min of mixing at any dosage of BrCl. The residual fraction of virus found at the lower concentrations of BrCl suggests either the presence of a resistant fraction of the poliovirus or it may simply reflect the depletion of an effective concentration of BrCl in the reaction vessel. To test the latter possibility, a fresh inoculum of poliovirus was added to a vessel containing a mixture of poliovirus and 0.15 mg/ℓ of BrCl which had been previously allowed to react for at least 10 min to ensure the completion of the inactivation reaction. The results showed that at least 1.5 logs of the freshly added virus inoculum was immediately inactivated within minutes and it was concluded that the residual fraction of virus remaining was not the result of the simple depletion of BrCl in the reaction vessel.

Effects of Nitrogen-Containing Compounds on BrCl

Nitrogenous compounds which are invariably present in natural and waste waters can react with chlorine to form chloramines which are less effective disinfectants than chlorine. Furthermore, the organic chloramines have been reported to be less effective disinfectants than the inorganic chloramines (Kruse 1969). To compare the interfering effects of nitrogen-containing

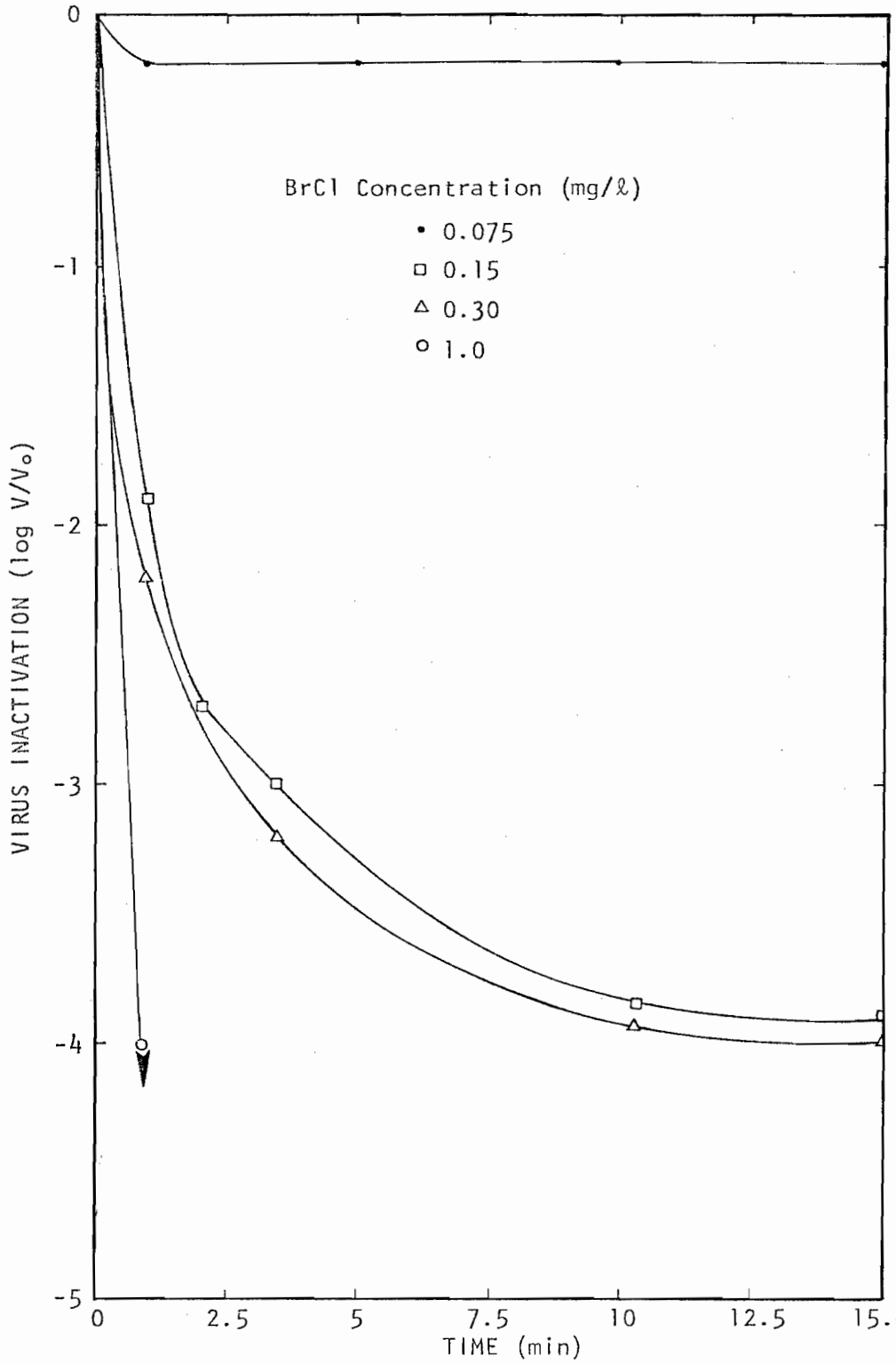

FIGURE 4. KINETICS OF POLIOVIRUS INACTIVATION BY VARIOUS CONCENTRATIONS OF BrCl

organic compounds on BrCl and chlorine, various concentrations of glycine were added to 0.15 mg/ℓ of BrCl and to 0.30 mg/ℓ of chlorine prepared in the nitrogen-free buffer at pH 7.0. Aliquots of poliovirus was then added to these solutions and mixed for 15 min at 25°C in the dark, after which the samples were immediately assayed for virus. The results in Figure 5-A show that $1.8 \times 10^{-5} M$ glycine completely inhibited the virus inactivating capacity of 0.3 mg/ℓ of chlorine, but only partially inhibited that of 0.15 mg/mℓ of BrCl. The results indicate that glycine interferes with the virus inactivating potential of chlorine to a greater extent than that of BrCl and suggests that organic bromamines are superior disinfectants to organic chloramines.

In corollary experiments, the interfering effects of an organic compound such as, glycine, and an inorganic compound such as, NH_4Cl, on the virus inactivating potency of BrCl were compared. Three mg/ℓ BrCl were added to the nitrogen-free buffer containing various concentrations of either glycine or NH_4Cl. Poliovirus was then added to these solutions mixed for 15 min and assayed for residual virus. The results (Fig. 5-B) show that while the presence of 1.8 to $9.0 \times 10^{-4} M$ of NH_4Cl had no demonstrable effect on the virus inactivating efficiency of BrCl, the presence of $3.6 \times 10^{-4} M$ of glycine markedly interfered with the inactivating capacity of 3 mg/ℓ BrCl. Thus, as was reported for chlorine, the virus inactivating capacity of BrCl is interfered to a lesser degree by NH_4Cl then by glycine and strongly indicates that organic bromamines are less effective disinfectants then inorganic bromamines.

Inactivation of Poliovirus in Sewage Effluent

The accumulated results thus far strongly suggest that BrCl is superior to chlorine in inactivating poliovirus suspended in simple aqueous laboratory reagents. To obtain evidence that BrCl is a superior virus inactivating agent to chlorine even when the virus is mixed in natural waste water, 50-mℓ aliquots of secondary sewage effluent, which had been previously clarified through a Millipore AP 20 filter, were seeded with poliovirus and then dosed with various concentrations of either BrCl or chlorine. The samples were mixed for 15 min at 25°C in the dark and assayed for virus. The results in Figure 6-A show that as the dose of BrCl and chlorine was increased from 1 to 3 mg/ℓ, inactivation of poliovirus increased to approximately the same

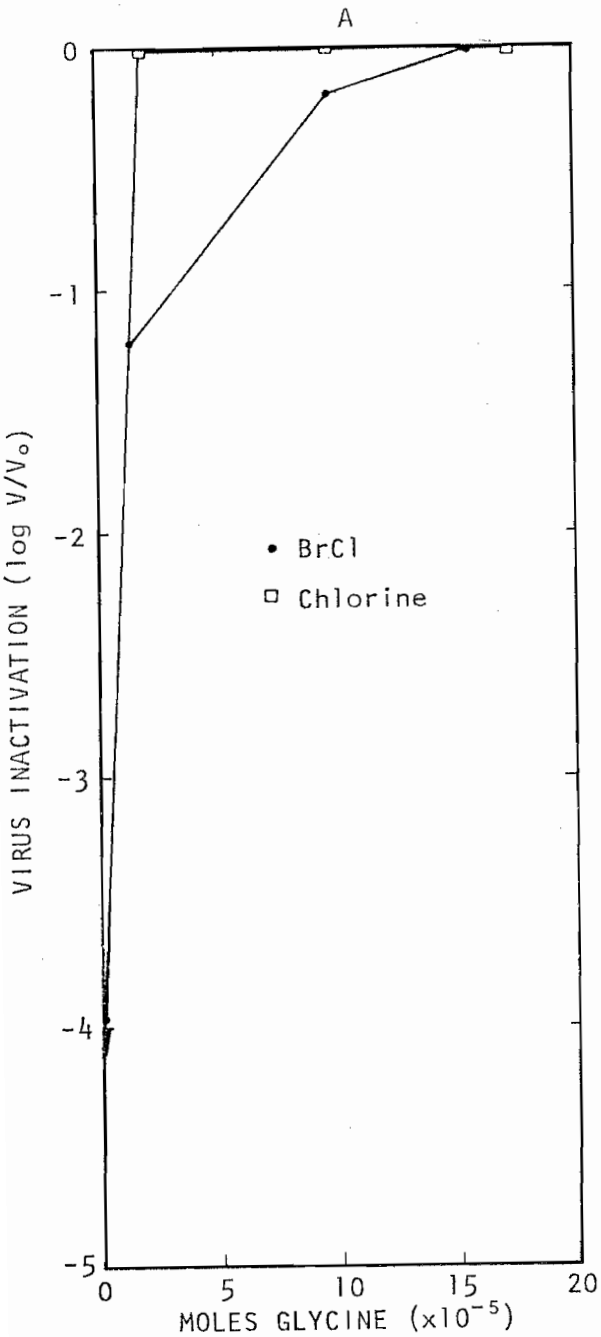

FIGURE 5-A. EFFECT OF VARIOUS CONCENTRATIONS OF GLYCINE ON POLIOVIRUS INACTIVATION BY 0.15 mg/ℓ BrCl VS. 0.3 mg/ℓ CHLORINE

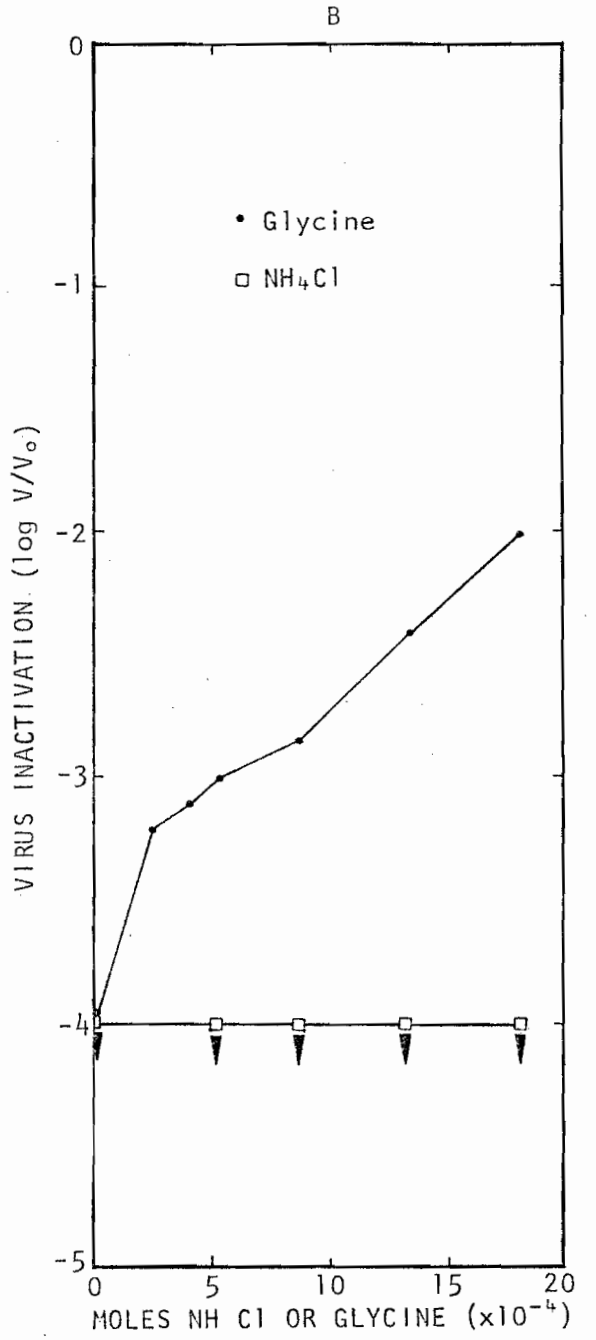

FIGURE 5-B. EFFECT OF VARIOUS CONCENTRATIONS OF GLYCINE VS. NH_4Cl ON POLIOVIRUS INACTIVATION BY 3.0 mg/ℓ BrCl

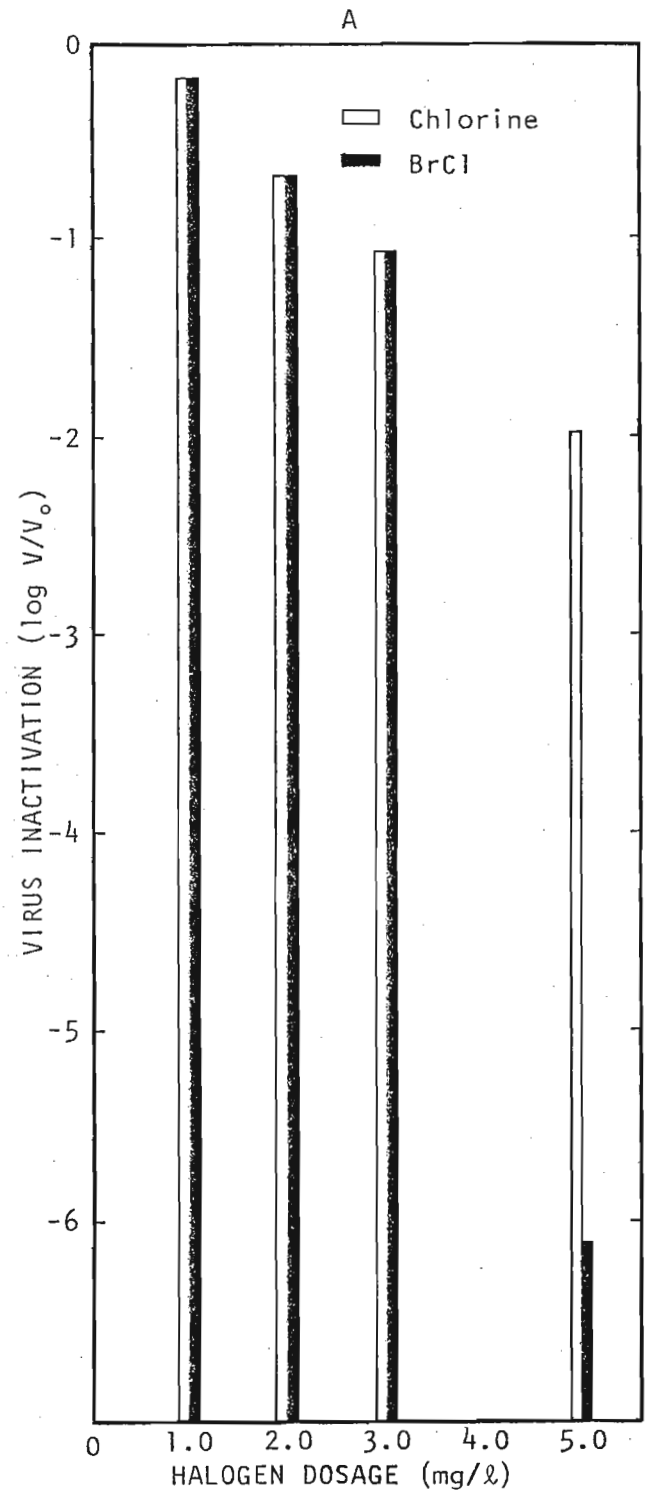

FIGURE 6-A. INACTIVATION OF POLIOVIRUS SUSPENDED IN AP-20 CLARIFIED, ACTIVATED SLUDGE TREATED SEWAGE EFFLUENT BY VARIOUS DOSES OF BrCl AND CHLORINE

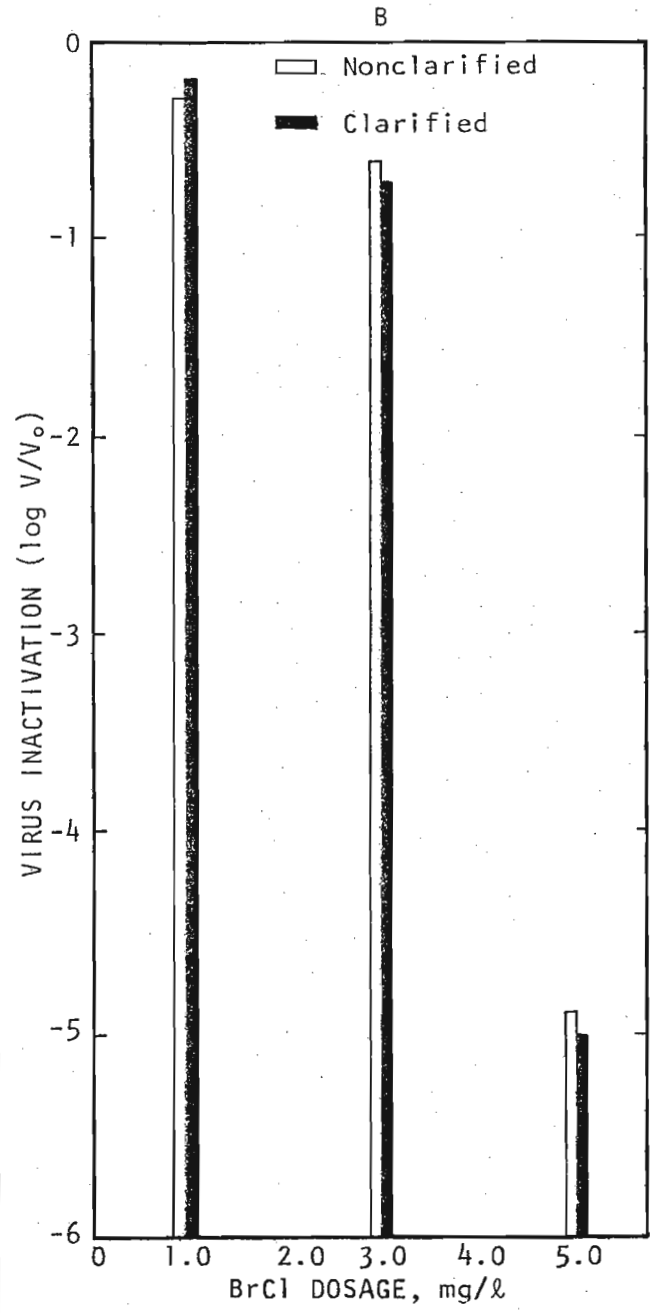

FIGURE 6-B. INACTIVATION OF POLIOVIRUS SUSPENDED IN NONCLARIFIED AND AP-20 CLARIFIED, ACTIVATED SLUDGE TREATED SEWAGE EFFLUENT BY VARIOUS DOSES OF BrCl

degree for both halogens (1 log reduction by 3 mg/ℓ halogen). However, while a dose of 5 mg/ℓ of BrCl inactivated greater than 5 logs of virus, an equivalent amount of chlorine inactivated approximately 2 logs of virus. These results show that BrCl is superior to chlorine as a viral disinfectant, even when the virus is mixed in natural sewage effluent. Since the effluent used was initially clarified of large particulate matter, the question was raised as to whether these particulate materials normally present in sewage effluents would significantly affect the disinfecting action of BrCl. In the following experiment poliovirus was seeded into 50-mℓ aliquots of both nonclarified (18 to 30 mg/ℓ suspended solids) and AP 20-clarified (6 to 15 mg/ℓ suspended solids) sewage effluents. These samples were then dosed with 1, 3, and 5 mg/ℓ of BrCl, mixed for 15 min at 25°C in the dark and immediately assayed for virus. The results in Figure 6-B show that BrCl was equally effective in inactivating poliovirus mixed in both clarified as well as nonclarified sewage effluents and strongly suggest that the large particulate matter in sewage will not interfere with the viral inactivating property of BrCl.

DISCUSSION

In the present investigation, the viral inactivating efficacy of BrCl was compared to chlorine under laboratory conditions and was found to be superior in several of the following respects: (1) BrCl was found to be twice as effective as chlorine in inactivating poliovirus; (2) the effectiveness of BrCl was not significantly affected by pH increases from 6 to 10 as has been reported for chlorine (White 1972); (3) nitrogen-containing organic and inorganic compounds, such as glycine and NH_4Cl, respectively, were less effective in interfering with the virus-inactivating action of BrCl than that of chlorine, thus supporting earlier reports that bromamines are more effective disinfectants than chloramines (Johnson and Overby 1971; Johnson and Sun 1975); (4) bromine chloride was found to be more effective than chlorine in the inactivation of poliovirus seeded in nonclarified, as well as clarified secondary sewage effluents, suggesting that BrCl would be highly effective when used to disinfect natural waste waters containing high concentrations of organic matter (28.5 mg/ℓ ammonia nitrogen) and suspended material (30 mg/ℓ suspended solids).

Like chlorine the kinetics of BrCl-inactivation of poliovirus in nitrogen-free buffer, followed essentially a first order reaction with almost all of the virus being inactivated within 2 min at a concentration of ≯1 mg/ℓ of BrCl. At lower concentrations (<0.3 mg/ℓ) of BrCl, the initial rapid rate of virus inactivation was followed by a slower rate of virus inactivation reflecting the presence of a residual fraction of viable virus. The reason for the residual fraction of virus was not completely clear. However, the experimental data indicate that it was not the result of a depletion of BrCl in the sample.

While the present data strongly suggests that BrCl should be seriously considered as a potential alternative to chlorine for the disinfection of drinking and waste waters, additional studies are required to establish the inactivating potency of BrCl against other pathogenic viruses, bacteria, fungi, and protozoa and, more importantly, whether the halogen can be effectively and readily utilized in the field without adversely affecting the environment. Studies along such lines are currently underway.

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