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TECHNICAL REPORT 8108

FREE AVAILABLE CHLORINE DISINFECTION CRITERIA FOR FIXED ARMY INSTALLATION PRIMARY DRINKING WATER

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report is a comparative evaluation of the kinetic inactivation by Free Available Chlorine (FAC) of the following disaggregated microorganisms, prepared to be free of extraneous chlorine demand. Bacteria tested were Escherichia coli (ATCC's 11229 and 23985) Salmonella typhimurium, Shigella boydii, and Vibrio cholerae. Viruses included f_2 coliphage, polioviruses I (LSc and Brunhilde), coxsackie B3, and echovirus 7. A chlorine-resistant yeast, Rhodotorula rubra, was also included for testing. Baseline studies

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were performed in chlorine demand-free water buffered to pH 5, 7, and 9; temperatures were 6°C and 22°C. Data revealed that disinfection efficiency was reduced during low temperature and high pH test conditions. All the enteroviruses exhibited anomalous 2-stage disinfection kinetics at pH 5, making disinfection of these viruses as fast or faster at pH 7 than at pH 5.

Further studies were done using synthetic interference water, designed to individually assess the effects of natural water constituents including color (organic acids), hardness (cations), and turbidity (inorganic) on FAC disinfection kinetics. The levels of constituents used did not exceed those specified by various regulatory agencies for primary drinking water. A final testing medium was composed of all the individual constituents run at 6°C and pH 9. Results indicated that 5 C.U. fulvic acid and 5 N.T.U. bentonite turbidity produced negligible effects on FAC disinfection at pH 5, 7, and 9. However, the presence of divalent cation (Mg or Ca) enhanced disinfection of enteroviruses and f_2 coliphage at pH 9 by 20 to 90 percent. Kinetics for the E. coli strains also showed an enhanced rate at pH 9. These high pH buffercation effects were also seen in the testing of the composited interference water. The cause of the 2-stage anomalous disinfection kinetics exhibited by the enteroviruses at pH 5 was shown to be due to chlorine-specific aggregation. Overall, data showed that the pathogenic viruses tested [polio I (Brunhilde), coxsackie B3, and echovirus 7] were much more chlorine-resistant than the vaccine strain of polioviruses. The bacterial pathogens (E. coli 23985, S. typhimurium, S. boydii, and V. cholerae) were more sensitive to FAC disinfection than the disinfectant-testing strain E. coli 11229.

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INTRODUCTION

For many years the US Army has been interested in and supported research on the Free Available Chlorine (FAC) disinfection of purified, monodisperse, waterborne microorganisms. Adequate disinfection of water supplies is of particular importance to the soldier, both under field conditions and at fixed installations. Even mild cases of diarrhea will cause impairment of troop effectiveness. Chlorine (Cl) has historically been the disinfectant of choice for potable water systems and is relatively cheap and effective when used properly. Many chlorine disinfection studies have been performed, but results of these studies are difficult to interpret because of uncontrolled conditions of microorganism aggregation and uncontrolled chlorine demand in the test systems.

Aggregation creates problems for disinfection, particularly with viruses. Virions on the interior of aggregates are shielded from the action of chlorine, as evidenced by skewed disinfection kinetics curves which tail off or are otherwise retarded. To complicate matters, aggregates of viruses do occur naturally; polioviruses and other enteroviruses may be excreted into a water supply as large aggregates of fecal origin.[1] In addition, enteroviruses will spontaneously aggregate at low pH levels or when subjected to variable ionic conditions.[2]

If the chlorine demand of a water supply is unknown or ignored, or if FAC resistance of microbial contaminants is unknown, chlorine may be ineffective in removing pathogens. Low chlorine dose treatments in waters with high chlorine demand may not only fail to destroy microorganisms, but can generate offensive tastes and odors and produce chlorinated hydrocarbons such as chloroform, which are held to be carcinogens.[3,4] Bacterial and especially viral pathogens may survive insufficient chlorination and cause sporadic outbreaks of gastroenteritis, hepatitis and other diseases. The incidence of waterborne disease has recently increased; a 50 percent increase was shown for 1976-1979 over 1971-1975.[5] Conversely, excessive use of chlorine must also be avoided because halogens are toxic. Excessive chlorine in potable water also produces unpalatable tastes and odors.

Proper assessment of chlorine disinfection under natural conditions requires an understanding of disinfection criteria under controlled optimum conditions so as to provide a baseline for comparison. The research was divided into three parts: 1) determination of the baseline inactivation times for a selected group of chlorine-resistant microorganisms in a totally chlorine demand-free, monodispersed state; 2) to ascertain, individually, the effects of water hardness, turbidity and organic pollution upon the FAC baseline disinfection rates; and 3) to determine FAC inactivation times for selected, Cl-resistant, microbial pathogens in a composited synthetic water at high pH and low temperature, constituting a "worst case" condition of post-treatment drinking water.

A literature survey provided rationale for the choice of microorganisms and test parameters. Baseline FAC disinfection times were to be determined at pH 5, 7, and 9 and at temperatures of 5° and 22°C, chosen to generate data comparable to that of other investigations in the field.

The choice of additives for the second part of the research was based on the work of Guter, Cooper, and Sorber.[6] Concentrations were determined by consulting and amalgamating US Environmental Protection Agency (EPA)[7] and World Health Organization standards.[8]

Synthetic colored water was the first constituent studied. Surface waters--lakes, rivers, and particularly swamps and bogs--contain variable amounts of organic matter derived mainly from decaying vegetable debris. This organic matter, largely humic and fulvic acids, imparts tastes and odors to potable water and can interfere in flocculation during water treatment.[9] Oliver and Visser[10] have shown that fulvic acid fractions of aquatic humic material are major chloroform precursors and that, due to low molecular weight and high surface activity of fulvic acids, these fractions are very difficult to remove in water treatment processes.[10]

Synthetic turbidity was the second constituent studied. The source of turbidity in water may be organic, as in sewage pollution, or inorganic, from clays and soils. It is well known that viruses adsorb to suspended solids. Moore et al.,[11] using bentonite clay, found that virus adsorption is independent of pH and that the adsorbed virus is infectious. Adsorbed viruses are also protected from FAC disinfection. Stagg et al.[12] showed that MS-2 bacteriophage adsorbed to bentonite required twice the disinfection time as the nonadsorbed control virus.

Synthetic hard water was the third constituent studied. Hard water is common in wells, lakes, and streams and is due to water contacting sedimentary rock strata, such as limestone. Hard water contains dissolved salts, usually calcium (Ca) and magnesium (Mg) carbonates or sulfates.[13] Although other cations may be present, Ca and Mg salts are the most abundant, comprising an average of 53 percent and 34 percent, respectively, of the total dissolved solids (TDS) in hard water.[13]

For the third part of this study, all the above individually tested constituents were combined to form a composited synthetic water as a test medium for disinfection.

The research goal was the establishment of disinfection criteria for primary drinking water of US Army fixed installations. The results of these studies are presented in this report.

MATERIALS AND METHODS

MICROORGANISMS

Microorganisms chosen for study, including bacteria, viruses and a yeast, were selected for chlorine resistance, ease of cultivation, and importance in worldwide water disinfection. Many important waterborne pathogens (e.g., agents of hepatitis, leptospirosis, and amebic dysentery) were not included because containment facilities were inadequate or cultivation was difficult. Table 1 lists the microorganisms tested and the rationale for their inclusion.

TABLE 1. MICROORGANISMS TESTED AND RATIONALE FOR INCLUSION

Microorganism	Reason for Testing
	Bacteria
Escherichia coli ATCC 11229	Disinfectant-testing strain[15,16] known for FAC resistance
Escherichia coli ATCC 23985	Enteropathogenic; infant diarrhea
Salmonella typhimurium ATCC 13311	Waterborne outbreaks of gastroenteritis; cause of food poisoning
Shigella boydii ATCC 9207	Bacillary dysentery; associated with outbreaks of gastroenteritis
Vibrio cholerae ATCC 14033 Biotype "El Tor"	Cholera; world-wide waterborne pathogen
	Yeast
Rhodotorula rubra ATCC 16639	Isolated from sewage sludge; FAC-resistant sewage yeast[17]
	Viruses
f_2 Coliphage	E. coli phage; frequently used in FAC testing[14,18,19,20]
Poliovirus I (LSc)	Vaccine strain, present in sewage,[21] frequently used in FAC testing
Poliovirus I (Brunhilde)	Highly virulent strain; responsible for paralysis and death; recognized reference strain[22]
Coxsackie B3 Virus	Enterovirus; may be responsible for gastroenteritis, aseptic meningitis; present in sewage[21]
Echovirus 7	Has been isolated from gastrointestinal tract; implicated in aseptic meningitis, diarrhea; present in sewage[21]

Bacteria

Five bacterial species were chosen for study. Escherichia coli 11229 and 23985, Salmonella typhimurium, and Shigella boydii were cultivated at 37°C on nutrient agar (Difco). Vibrio cholerae was grown at 37°C on nutrient agar supplemented with 0.5% NaCl. Assay of these bacteria was performed by spreading 0.1 or 0.2 mL inoculum on the agar surface of a 100-mm petri plate, followed by incubation at 37°C for 24 hr. Colonies were counted and calculated as colony-forming units (CFU) per mL.

Viruses

One bacteriophage and four enteric viruses were chosen for study. The f_2 bacteriophage was produced and assayed in E. coli K-13 according to the method of Loeb and Zinder.[14] Plaque assay for f_2 virus was by soft agar overlay at 35°C. Plaques were counted after 24 hr and expressed as plaque-forming units (PFU) per mL.

Poliovirus I (Brunhilde and LSc strains) and coxsackie B3 virus were produced in Hela cell culture at 37°C using Eagles medium (Grand Island) supplemented with 10 percent newborn calf serum (Biofluids) and 50 µg/mL gentamycin (Schering).

Echovirus 7 was produced in Buffalo green monkey kidney (BGM) cell cultures, utilizing medium 199 with 10% newborn calf serum and 50 µg/mL gentamycin.

All four enteroviruses were plaque-assayed on their respective cell monolayers using media solidified by 0.9 percent ionagar (Difco) on 35 mm 6-well plastic plates (Linbro-Costar), and incubated at 37°C for 48 hours. Plaques were visualized after a 1-hour application of 3 percent neutral red (Gibco) in a 1X solution of Hank's Balanced Salt Solution (Grand Island).

Yeast

Rhodotorula rubra, ATCC 16639, is a chlorine-resistant yeast found in wastewaters. It was originally provided by Dr. Englebrecht of the University of Illinois at Urbana. R. rubra was grown and assayed on the yeast-malt extract agar (YMA) of Surucu[17] at 25°C. Colony counts were made 3 to 4 days after inoculation and were expressed as CFU/mL.

PREPARATION OF CHLORINE DEMAND-FREE MICROBIAL STOCKS

Bacteria and Yeast

Each bacterial species was initially grown as a broth culture for 24 hr. One milliliter of the 24-hr culture was placed on each of 12 100-mm petri dishes containing the proper solid medium. Luxuriant growth occurred in 24 hr. The growth was scraped from the surface of the agar and placed in a 50-mL centrifuge tube containing chlorine demand-free 0.05 M phosphate buffer (pH 7). The tube was capped, agitated, and centrifuged at 8,000 rpm in a Sorvall model RC2-B centrifuge. The pellet was resuspended in chlorine demand-free phosphate buffer and spun again at 8,000 rpm. This procedure was repeated twice. The final pellet, free from extraneous chlorine demand, was

brought up to volume with phosphate buffer such that the titer of each stock was approximately 10^9 CFU/mL.

R. rubra was initially grown as an agar slant. Growth was removed and placed in 5-mL chlorine demand-free 0.05 M phosphate buffer. This was used to inoculate plates for chlorine demand-free yeast production. From this point, the procedure for R. rubra was the same as for the bacteria.

Viruses

All viruses were prepared to be chlorine demand-free and monodisperse by the method of Sharp and Johnson,[23] which employs differential ultracentrifugation and a freon extraction. The final preparation of each virus was diluted in chlorine demand-free 0.05 M phosphate buffer to contain approximately 10^8 PFU/mL.

IDENTIFICATION OF VIRUSES

All enterovirus stocks used in this study were produced from previously existing laboratory stocks. An identity check was performed on each before use. Neutralization tests were performed with each virus using specific antisera (American Type Culture Collection, ATCC). Identities of stocks were confirmed.

AGGREGATE CHECK

All chlorine demand-free stocks of test microorganisms were checked for the presence of aggregates. Bacterial and yeast stocks were examined by light microscopy and were found to be virtually aggregate-free. The enteroviruses and f_2 coliphage chlorine demand-free stocks were examined for aggregates by sucrose density gradient analysis, using a method similar to that of Sharp and Johnson.[23] A Beckman SW 25.1 swinging bucket rotor with three buckets each containing 34 mL was used. Concentration range of sucrose in each gradient was 5 to 30 percent. One milliliter of each virus suspension was layered onto sucrose gradients. Spin time in the Beckman L-50 ultracentrifuge was 1.5 hr at 25,000 rpm. After centrifugation, approximately 20 fractions were collected from each gradient tube, and each fraction was assayed for live virus. All the enteroviruses and f_2 coliphage assays produced similar (count distributions in the gradient) results. In each case, a single, large central peak was formed which contained 99.9 percent of the input virus. Young and Sharp[1] found by electron microscopy that these central fractions contain nearly all singles and some pairs of virions.

REAGENTS--CHLORINE DEMAND-FREE WATER AND GLASSWARE

All chlorine kinetic inactivation experiments were conducted using chlorine demand-free water, glassware, and test organism suspensions. Glassware was acid-washed in sulfuric acid-dichromate and then washed conventionally. Cleaned glassware was soaked for a minimum of 48 hr in a 10 mg/L chlorine bath, rinsed with chlorine demand-free water, and sterilized in a forced hot air oven (Blue M). Water and buffers were made chlorine demand-free by adding sufficient HOCl (Clorox^R) to produce a 1-mg/L FAC residual after 48-hr contact. Solutions were dechlorinated by broad-spectrum ultraviolet light (ACl-Hanovia) or by sunlight. Chlorine demand-free water was stored in 5-gal glass

bottles and dispensed via siphons as described by Cuter et al.[6] Air entering the siphon system was scrubbed by a H_2SO_4 trap designed to exclude ammonia and other laboratory contaminants.

BUFFERS

Buffers chosen have been commonly used in chlorine disinfection studies. Stock buffers were prepared at 0.05 M in chlorine demand-free water.

pH 5

Stock acetate buffer was prepared by combining 4.9 g $NaC_2H_3O_2$ and 1.43 mL glacial acetic acid. Volume was brought to 1 liter with chlorine demand-free water.

pH 7

Phosphate buffer was prepared by combining 5.29 g KH_2PO_4 and 7.10 g Na_2HPO_4 in 1 liter (final volume) chlorine demand-free water.

pH 9

Borate buffer was prepared by adding 19.067 g $Na_2B_4O_7 \cdot 10H_2O$ to 1 liter chlorine demand-free water. Working strength of these buffers was 0.005 M. An alternate pH 9 buffer was phosphate-carbonate, described by Floyd and Sharp.[24] This was prepared 10X in two parts: 10X phosphate was made by adding 13.61 g KH_2PO_4 to 1 liter chlorine demand-free water; 10X carbonate was prepared by adding 4.5 g anhydrous Na_2CO_3 to 1 liter chlorine demand-free water. The working buffer consisted of 32 mL 10X phosphate plus 68 mL 10X carbonate in 1 liter chlorine demand-free water. The working strength of this buffer was 0.0027 M. The pH of this and the other buffers was adjusted by 0.1 N NaOH or 0.1 N HCl.

CHLORINE SOLUTIONS

Stock chlorine was prepared by adding 2 mL commercial chlorine bleach (Clorox^R) to 1 liter chlorine demand-free water. This resulted in a 100 mg/L stock, which was stored in low-actinic glass bottles for preservation of the chlorine residual.

CHLORINE DETERMINATION

Free available chlorine and total combined chlorine determinations were performed routinely on each test mixture by amperometric titrations (Fischer-Porter). The syringaldazine method[6] was used for a quick check of solutions undergoing dechlorination.

NEUTRALIZER

Sodium thiosulfate solutions (0.02 N) were used to simultaneously neutralize chlorine and dilute samples for assay. Neutralizer was prepared in nutrient broth for use with the bacterial and coliphage samples. Neutralizer for enteroviruses was made in 1X Dulbecco's phosphate-buffered saline (PBS). R. rubra samples were neutralized by sodium thiosulfate in distilled, deionized water.

SYNTHETIC INTERFERENCE WATERS

Synthetic Organically Contaminated Water

Fulvic acid was prepared from sphagnum peat according to the method described by Narkis and Rebhun.[9] The final product, containing clarified, acid-soluble fulvic acids, was evaporated under vacuum (24 mm Hg) at 40°C and lyophilized under vacuum (30 mm Hg) at 30°C. The dried product was ground to a powder and weighed. A 100X stock solution of fulvic acids was made by dissolving 200 mg in 1 liter Cl demand-free water. Color determinations were made using the platinum-cobalt test.[25] Working solutions contained 5 color units (C.U.), the maximum permitted by EPA drinking water standards.[7]

Synthetic Hard Water

Stock solutions of $CaCl_2$, $MgCl_2$, KCl, and NaCl were prepared at 5 M, 1 M, or to contain 25 g/L of the cation in question. All 100X stocks were prepared in chlorine demand-free water and were sterilized by passage through a 0.22-μ membrane (Millipore).

Synthetic Turbid Water

Bentonite clay was chosen to simulate natural inorganic turbidity of drinking water. A stock suspension was prepared by adding 500 mg bentonite (Fisher Scientific-USP Grade) to 1 liter chlorine demand-free water. Turbidity was measured in a Hach Model 2100A turbidimeter with results expressed as nephelometric turbidity units (NTU). Usually a 1:20 or 1:50 dilution of the stock preparation was necessary to make test solutions of 5 NTU. Dilutions were done in buffered chlorine demand-free water, and pH was the same as the test run.

Synthetic Composite Installation Water

All the above mentioned additives were combined to make a composite test water, which was buffered at pH 9, contained 5 C.U., 250 mg/L hardness, and 5 NTU turbidity, and was tested at 6°C.

EXPERIMENTAL DESIGN

Baseline Determinations (General Procedure)

Research requirements are to determine minimum chlorine dosages and contact times needed to inactivate 4 logs of microorganisms. To accomplish this, inactivation rates of the basic test groups of microorganisms and selected pathogens in well defined, minimally buffered waters were observed.

All baseline disinfection experiments were conducted at ambient temperature (ca 22°C) and at 6°C ± 1°C. FAC concentrations ranged from 0.1 to 5.0 mg/L. One-liter chlorine demand-free beakers contained 400 mL of test solution at pH 5, 7, or 9. Synchronous mixing and temperature control were provided by a thermostat-controlled Frigidflow bath circulator connected by insulated copper tubing to a plastic pan atop a six-place multiple stirrer (Labline).

Previously titrated chlorine demand-free microorganism stocks were diluted with 0.05 M chlorine demand-free phosphate buffer to contain 1 to 2 x 10^7 organisms/mL. One milliliter of this suspension was seeded into 400 mL of test mixture at the start of each run, resulting in an organism concentration of approximately 5 x 10^4 organisms/mL. Mixing was begun with a mixing speed sufficient to bring the vortex to the bottom of the beaker. Chlorine was added at time zero (t=0) and samples were withdrawn at various time intervals. All samples, including nonchlorinated controls, were simultaneously neutralized and diluted for assay after being withdrawn, the moment of neutralization being the exact time specified for the sample. (Usually 4 seconds were required to withdraw the sample from the beaker and neutralize it.) Neutralized samples were immediately placed on ice. Assay was performed the same day.

Controls were always included in each experimental run. Temperature and chlorine measurements were made before and after every test run, and a direct measure of the input microorganism accompanied every assay. Control beakers included buffered, nonchlorinated, and neutralizer (chlorinated) control beakers. Stock chlorine residuals were measured each test day.

Synthetic Interference Water (General Procedures)

Experiments were conducted with the basic test group of microorganisms and various types of interference water. All experiments using interference water were conducted at 6°C ± 1°C. Each additive (color, turbidity, hardness) was tested separately at pH 5, 7, and 9. Before use, each additive, as a working solution, was tested for chlorine demand.[6] Two levels of testing were performed for any additive found to consume chlorine. In one set, chlorine was prereacted with the test solution so that the chlorine demand of 30 min was satisfied and an appropriate amount of FAC was produced. In the other set, chlorine was added, without regard for chlorine demand, at t=0.

Synthetic composite installation water was also tested at two chlorine levels. In one set, a predetermined quantity of chlorine, sufficient to produce the desired FAC residuals at 30 min, was added to the test water at t=0. In the other set, chlorine was added without regard for chlorine demand at t=0.

Input, temperature and buffer controls for synthetic composite installation water were similar to those for the baseline experiments. Chlorine was measured immediately after each run but neutralizer controls were not included. A nonchlorinated test water control was added.

Data Collection and Handling

Triplicate plate counts of colonies or plaques of surviving microorganisms generated by kinetic inactivation experiments were averaged and expressed as CFU or PFU per 400 mL. These values were compared with values from the appropriate nonchlorinated control beaker. Plots were drawn of log percent survivors versus sampling time to determine the 99.99 percent inactivation time.

A minimum of two runs were performed for each microorganism and test parameter. Sufficient runs were conducted so that data generated represented

unequivocal points for kinetic inactivation curves. Except for enterovirus data from pH 5 studies, all kinetic inactivation plots were first-order.

Dose-response curves were created by plotting the time required to inactivate 99.99% of input microorganisms (data from kinetic inactivation plots) versus the chlorine dose.

RESULTS AND DISCUSSION

BASELINE DISINFECTION

Bacteria

Dose-response curves were developed from data generated by baseline inactivation experiments. Figure 1 shows the dose-response of E. coli 11229 to FAC disinfection. Data were collected at 6°C and pH 5, 7, and 9 and at 22°C and pH 9. In nearly all the dose-response curves drawn, the shape of the curve is initially linear, depending on FAC concentration and organism tested, followed by a dramatic tail-off. At 0.1 mg/L FAC, pH 9 and 6°C, the time required to kill 99.99 percent of the input E. coli is 1.4 hr.

Other bacterial strains tested revealed much greater sensitivities to FAC at all pH levels. Preliminary studies performed at 22°C, pH 5, 7, and 9 and at 6°C, pH 5 and 7, showed that few CFU survived when tested under these conditions. Figure 2 shows the comparative dose-responses of V. cholerae, S. typhimurium, Sh. boydii, E. coli 23985 (enteropathogenic), and E. coli 11229 to FAC disinfection at pH 9 and 6°C, and Table 2 presents the times required for inactivativation of 99.99 percent of input bacteria by 0.20 mg/L FAC . The experimental conditions were 30 min contact time at pH 9 and 6°C. The cholera bacterium is the most sensitive to chlorine, while E. coli 11229 is the most resistant. The bacterial pathogens were all more susceptible to FAC inactivation than the nonpathogenic E. coli 11229. All strains tested were inactivated easily by 0.2 mg/L FAC or less within 30 min. However, since viruses as well as bacteria are present in potable water supplies, the fact that bacteria are easily destroyed by low FAC levels does not assure safety.

TABLE 2. INACTIVATION OF ENTEROBACTERIA BY 0.20 mg/L FAC AT pH 9 AND 6°C

Microorganism	99.99% Inact Time (min)
E. coli 11229	13.1
E. coli 23985	4.9
S. boydii	3.4
S. typhimurium	2.3
V. cholerae	1.1

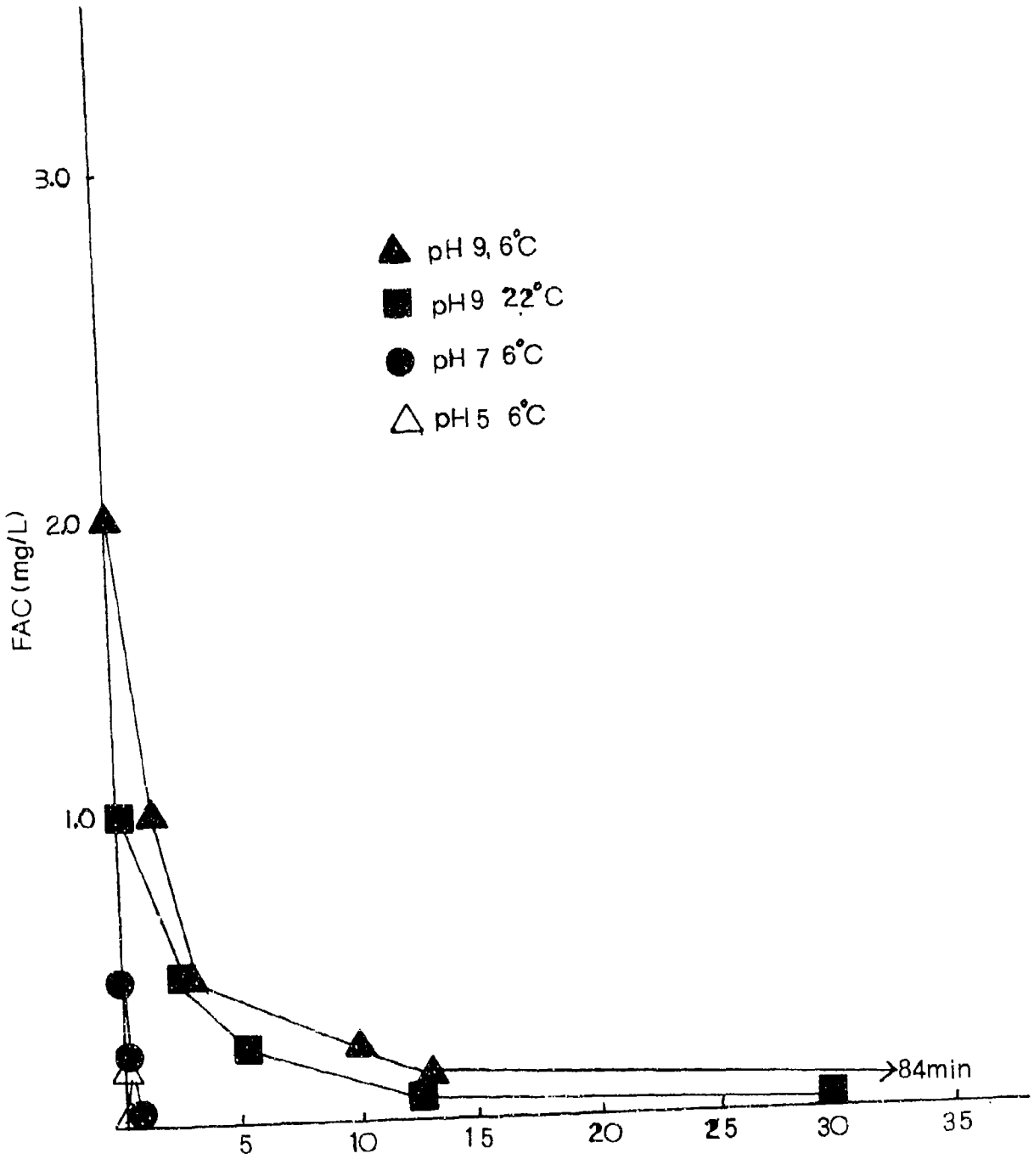

Figure 1. Dose-Response of E. coli 11229 to FAC Disinfection at pH 5, 7, and 9 at 6° and 22°C.

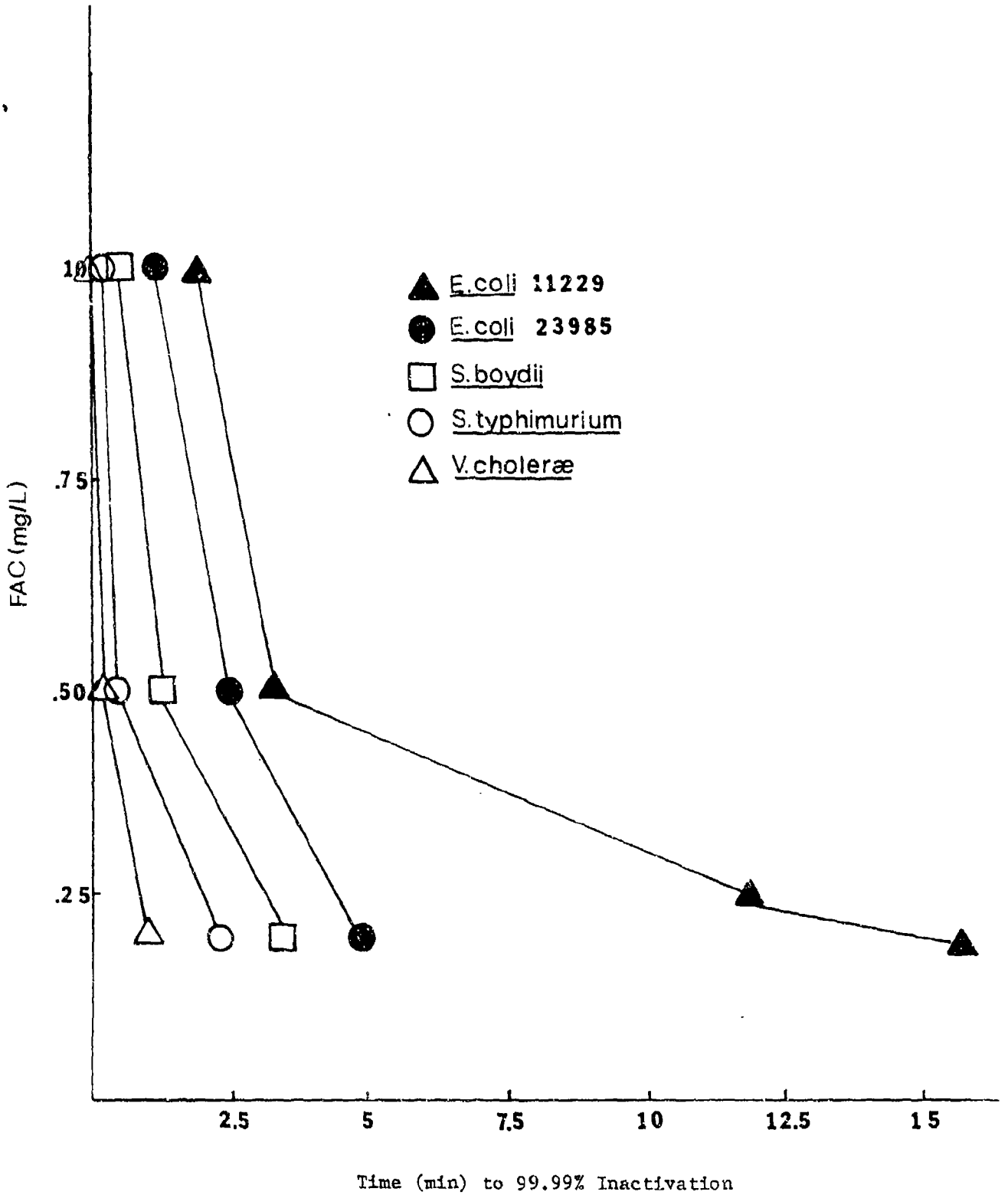

Figure 2. Comparative Dose-Responses of Enterobacteria to FAC Disinfection at pH 9 and 6°C.

Yeast

Figure 3 illustrates the dose-response of R. rubra to FAC at pH 5, 7, and 9 at 6° and 22°C. Satisfactory disinfection (99.99 percent in 30 min) required at least 1.0 mg/L FAC at pH 5 and 22°C, at least 2.0 mg/L FAC at pH 5 and 6°C, and at least 2.0 mg/L FAC at pH 7 and 6°C. There was no 99.99 percent inactivation of the yeast at pH 9 within 30 min by any FAC levels tested (up to 5 mg/L FAC) at either 6° or 22°C.

Yeasts commonly found in sewage are nonpathogenic to man. In fact, many yeasts known to be human pathogens are opportunistic; they exist among the normal flora of mucus membranes, such as those of the mouth, and become pathogenic only when the host experiences poor resistance, is already infected by a virulent organism, or lacks proper immune response, as in cancer therapy. Other pathogenic yeasts and fungi are transmitted to man by airborne spores or by direct contact with an infected lesion. None of these has been implicated as a waterborne pathogen.[26] Study results indicate that R. rubra is highly resistant to FAC disinfection. Although Englebrecht et al.[27] suggest using sewage-borne yeasts as disinfection indicators, their resistance to FAC inactivation would result in unnecessarily large chlorine applications.

Viruses

Enteroviruses and f_2 coliphage were, as a group, much more resistant to FAC at all pH and temperature levels. Figure 4 shows the dose-response of f_2 coliphage at pH 5, 7, and 9 at 6° and 22°C. The f_2 virus response appears to show a linear decrease from 5 mg/L to 2 mg/L FAC followed by a tailing off of disinfection efficiency at all pH and temperature levels. However, in tests at 6°C, pH 7 and 9, the curve levels off; given a 30-min contact time, f_2 virus is not satisfactorily disinfected by 0.2 mg/L FAC at either pH 7 or 9. As with the bacteria, disinfection of viruses is most effective at pH 5, 22°C.

Figure 5 illustrates the dose-response of poliovirus I (LSc) at pH 5, 7, and 9 at 6° and 22°C. Again, the response proceeds with an initial linear decrease down to 2 mg/L FAC, after which a tailing off occurs, seen most dramatically at 6°C. (A minimum of 0.25 mg/L FAC at 22°C and 0.50 mg/L FAC at 6°C is required to inactivate poliovirus I at pH 9 in 30 min.) An important feature to be noted is that disinfection proceeds faster at pH 7 than at pH 5. This effect will be discussed later in detail.

Figure 6 shows the dose-responses of f_2 coliphage, polio I (LSc), polio I (Brunhilde) and coxsackie B3 viruses to FAC disinfection at pH 5. Data indicate that poliovirus I (Brunhilde) is the most resistant while the resistances of polio I (LSc) and coxsackie B3 virus to FAC are equal at pH 5. The f_2 coliphage is the most sensitive to FAC at this pH value.

Figure 7 illustrates the comparative dose-responses of the enteroviruses and f_2 coliphage to FAC disinfection at pH 7. Poliovirus I (LSc) and f_2 coliphage are equally sensitive to FAC at this pH, followed by coxsackie B3 virus. Poliovirus I (Brunhilde) is the most resistant.

Figure 8 shows the comparative resistances of these viruses to FAC disinfection at pH 9 in borate buffer. Echovirus 7 data are included in this figure. Coxsackie B3 is, by far, the most FAC-resistant virus at this pH

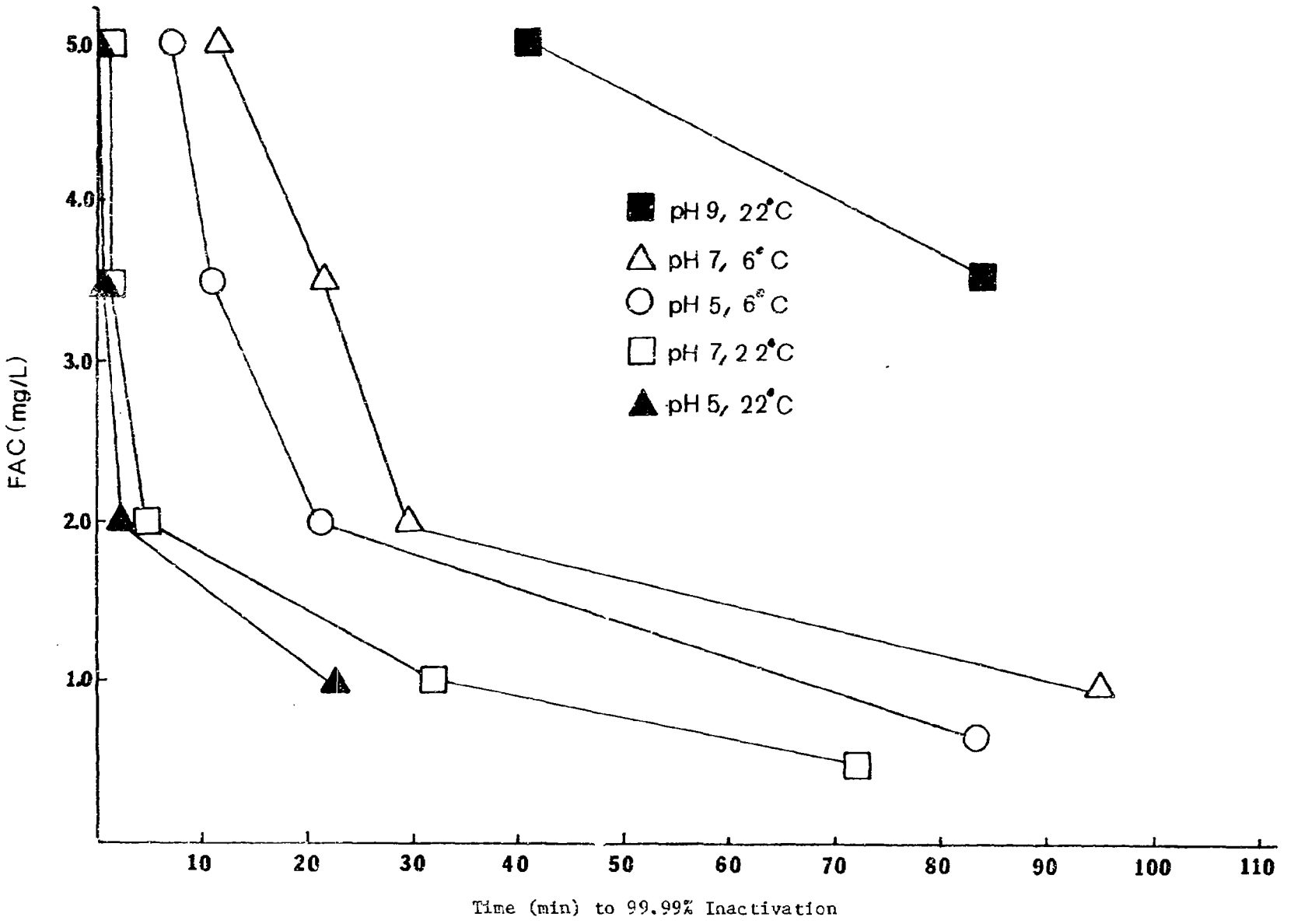

Figure 3. Dose-Response of R. rubra to FAC at Disinfection pH 5, 7, and 9 at 6° and 22°C

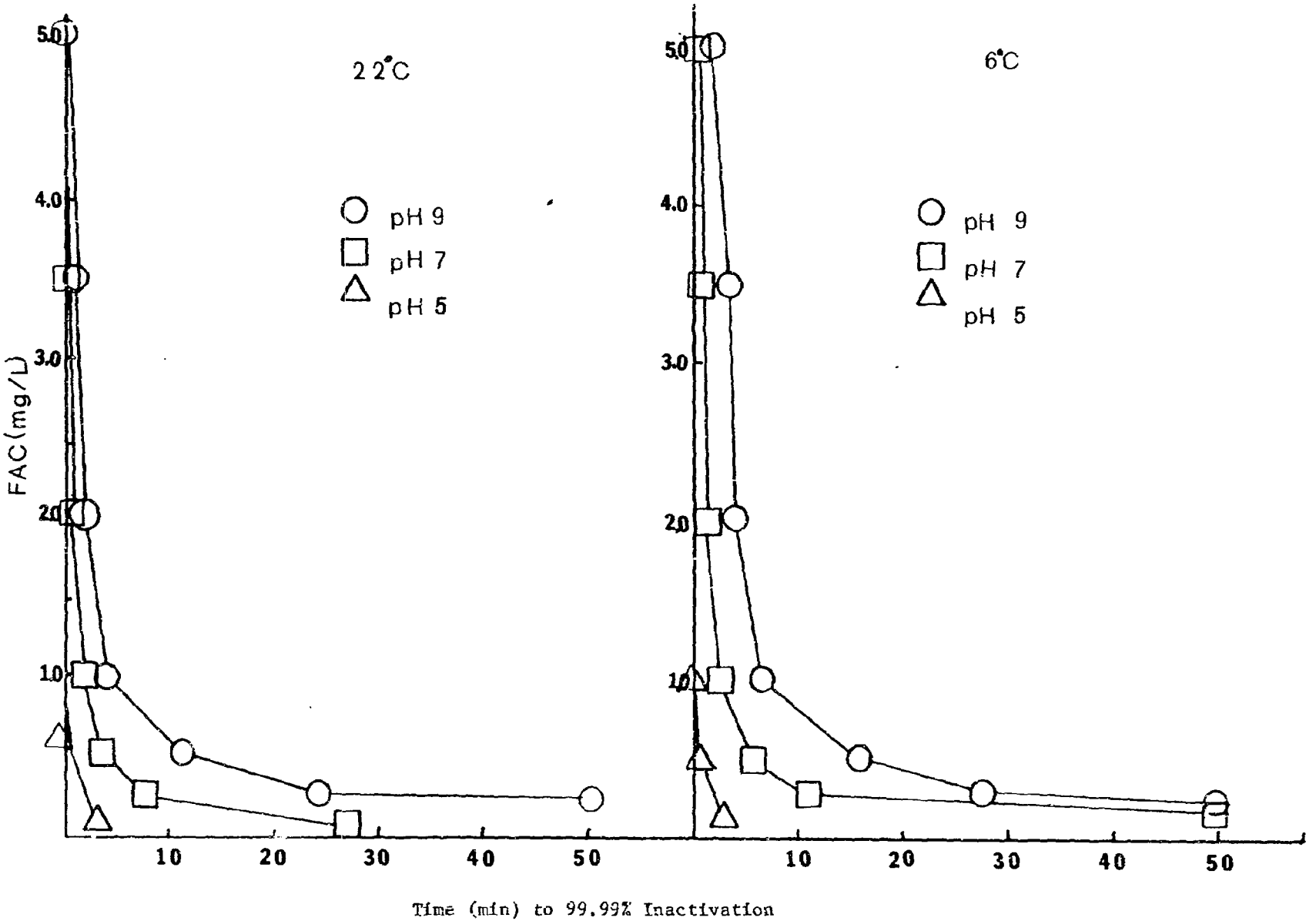

Figure 4. Dose-Response of f_2 Coliphage to FAC Disinfection at pH 5, 7, and 9 at 6° and 22°C.

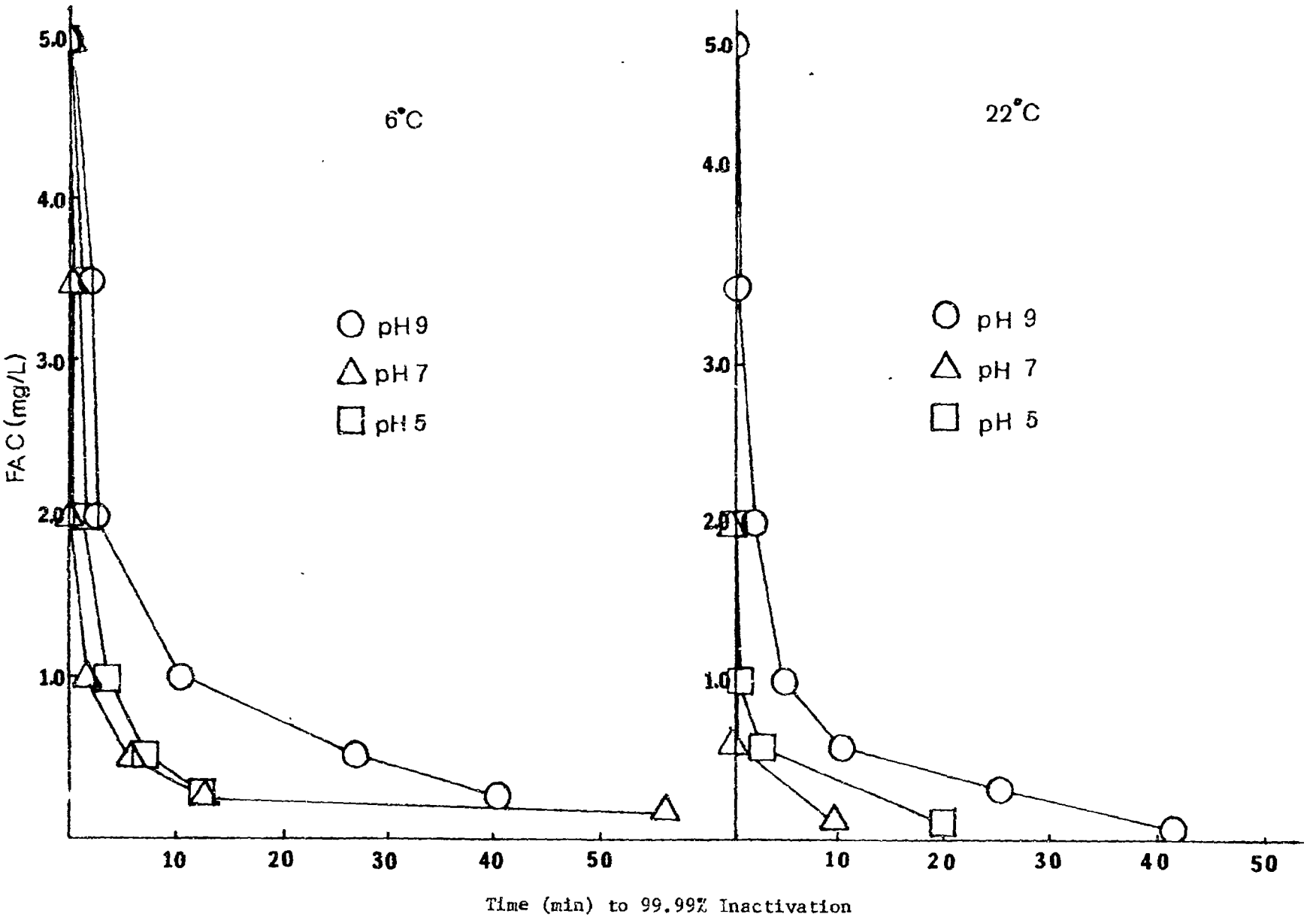

Figure 5. Dose–Response of Poliovirus I (LSc) to FAC Disinfection at pH 5, 7, and 9 at 6° and 22°C.

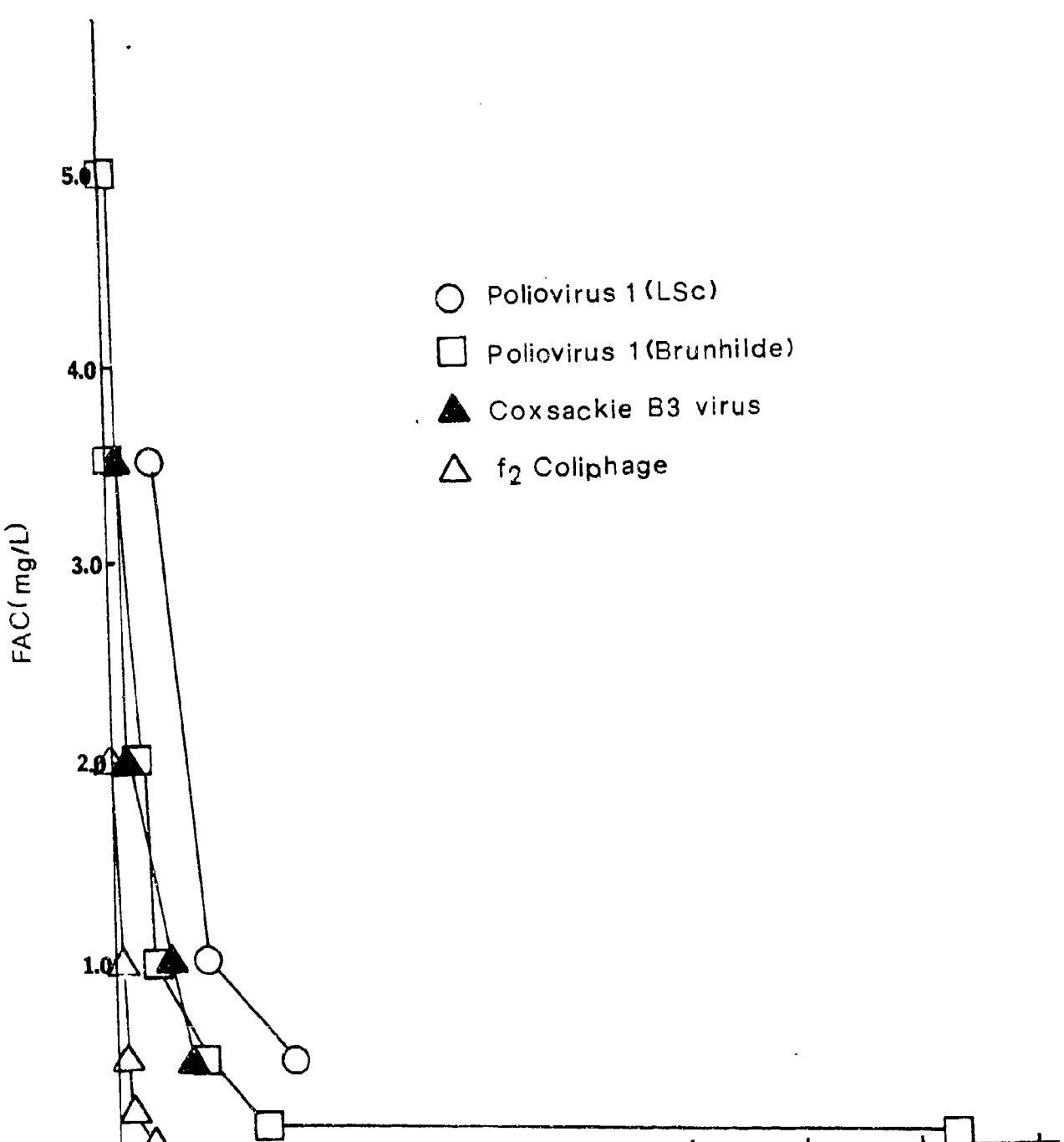

Figure 6. Comparative Dose-Responses of Enteroviruses and f_2 Coliphage to FAC Disinfection at pH 5 and 6°C.

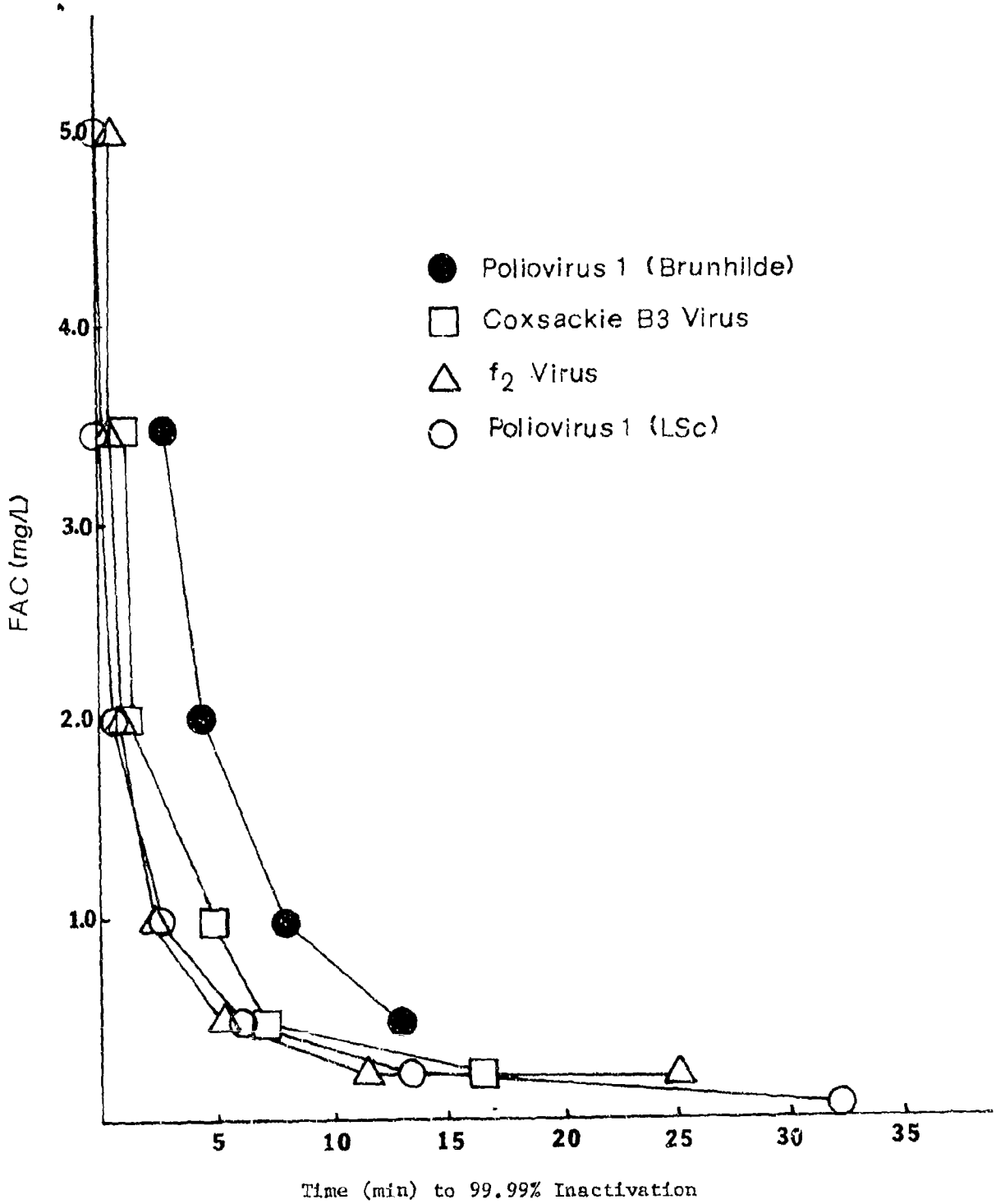

Figure 7. Comparative Dose-Responses of Enteroviruses and f_2 Coliphage to FAC Disinfection at pH 7 and 6°C.

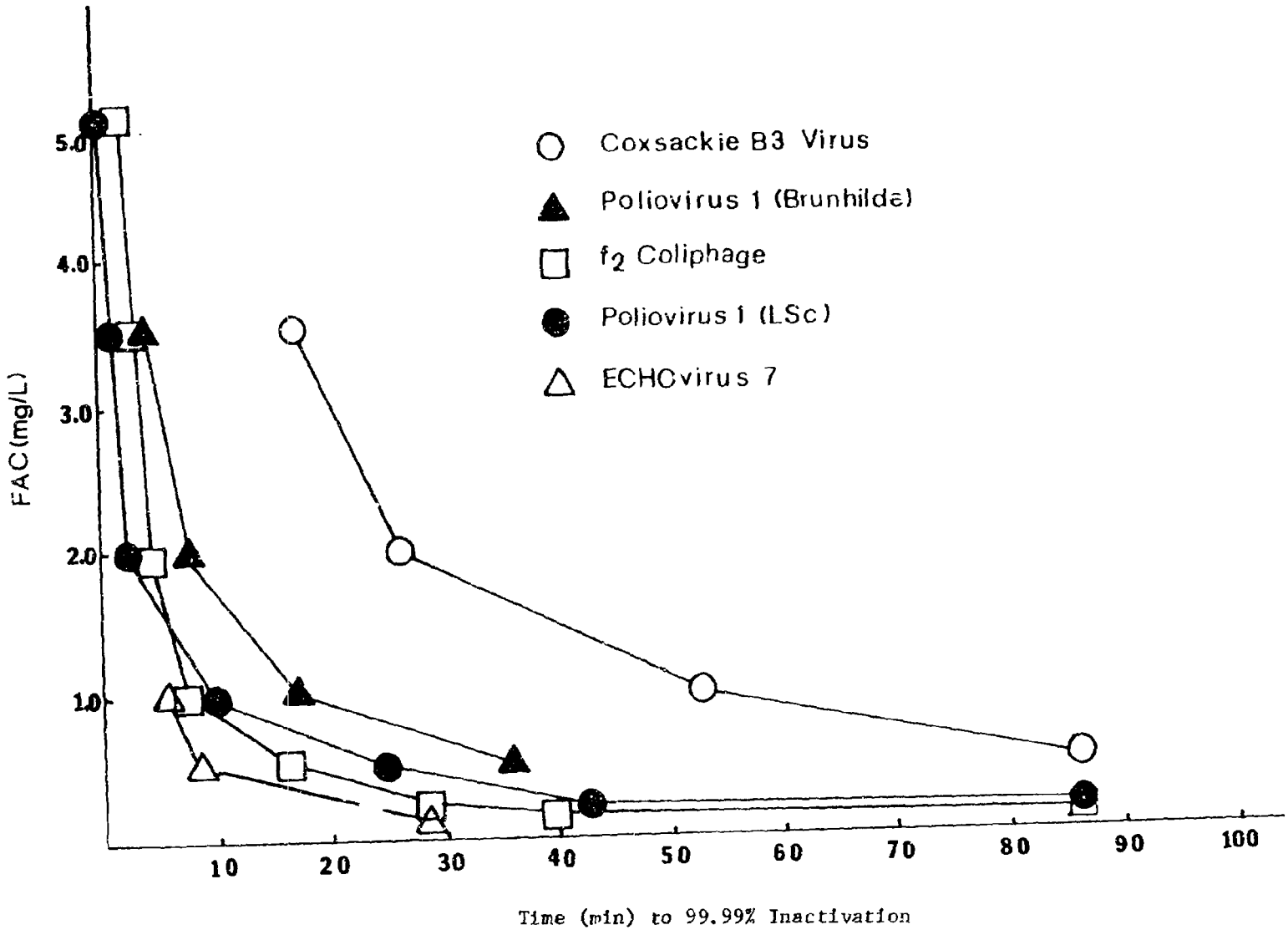

Figure 8. Comparative Dose-Responses of Enteroviruses and f_2 Coliphage to FAC Disinfection at pH 9 and 6°C.

while echovirus 7 is the most sensitive. The order of resistance at pH 9 is echovirus 7 < f_2 coliphage < polio I (LSc) < polio I (Brunhilde) < coxsackie B3.

Table 3 shows the comparative resistances of f_2 coliphage, polioviruses I (vaccine and pathogenic), and coxsackie B3 virus to FAC disinfection at 6°C in 0.0027 M phosphate-carbonate (P-C) buffer at pH 9 compared with resistances in borate test solutions. Although only preliminary work has been done using P-C buffer, the data clearly show that disinfection proceeds faster in this buffer In particular, the inactivation time of coxsackie B3 virus was reduced an average of 64 percent over borate by using P-C buffer. Other viruses showed 22 to 30 percent reductions in inactivation time.

TABLE 3. COMPARATIVE RESISTANCE OF ENTEROVIRUSES AND f_2 COLIPHAGE TO FAC DISINFECTION IN PHOSPHATE-CARBONATE AND BORATE BUFFERS AT pH 9 AND 6°C

Virus	FAC Dose (mg/L)	99.99% Inactivation Times		% Decrease in Inactivation Time Effected by P-C over Borate
		Borate (min)	P-C (min)	
f_2 Coliphage	1.00	7.0	5.5	28.6
	0.50	16.0	11.5	28.1
Poliovirus I (LSc)	1.00	11.5	9.0	21.7
	0.50	27.0	16.5	38.9
Poliovirus I (Brunhilde)	1.00	17.5	13.7	21.7
	0.50	36.0	28.1	21.9
Coxsackie B3	3.50	18.5	7.0	62.2
	1.00	55.0	19.0	65.5

Virus aggregation cannot be ruled out as an explanation for the increased disinfection time observed in borate buffer as compared to P-C buffer. The large dilution factor renders this less probable, however, as Floyd and Sharp[28] have suggested that aggregation of enteroviruses is unlikely at high dilution. Furthermore, the FAC disinfection kinetics for viruses in borate buffer were linear (first-order), indicating dispersed virus, although Galasso and Sharp[29] reported that a die-away of viruses resembling a first-order curve might be due to extensive aggregation with just the right distribution frequency. Whatever the explanation, it is clear that the true inactivation times of dispersed virus at pH 9 were found using P-C buffer.

Table 4 summarizes the virus baseline data, listing all 99.99 percent end points found for the various parameters. This table demonstrates the following:

1. Both vaccine and pathogenic polio I and coxsackie B3 viruses are inactivated as fast or faster at pH 7 as at pH 5. This finding will be discussed further.

2. The efficacy of P-C buffer over borate as test medium is shown.

3. Disinfection at 22°C is roughly 1.5 times faster than at 6°C.

4. With the exception of echovirus 7, none of the viruses can be confidently destroyed by 0.2 mg/L FAC at 6°C at any pH. The data show that the least resistant virus (echovirus 7) is approximately 3 times as resistant to FAC as the most FAC-resistant bacterium (E. coli 11229).

Results of the baseline disinfection study clearly indicate 0.2 mg/L FAC, although adequate for killing bacteria, are totally inadequate for inactivating the viruses used in this study. The presence of bacteria in potable water, particularly fecal bacteria, implies the presence of enteroviruses as well. If chlorine residuals are provided to kill bacteria, the residuals should be high enough to kill viruses as well.

SYNTHETIC INTERFERENCE WATER

Microorganisms tested in the various types of interference water were E. coli 11229, f_2 coliphage, R. rubra, and poliovirus I (LSc).

Organic Contaminant

When tested at pH 5 and 7, 99.99 percent of E. coli 11229 did not survive more than 7 sec at any of the FAC levels tested. Table 5 presents results of testing E. coli 11229 at pH 9, which indicate that the 99.99 percent inactivation times for E. coli were not increased by the chlorine demand of fulvic acid. Disinfection in the prereacted beakers caused faster inactivation than in the appropriate chlorine demand-free controls, faster, even, than those inactivation times that the initial FAC concentration (at t=-50 min) would yield under chlorine demand-free conditions. Inactivation times for non-prereacted solutions were no greater than matching chlorine demand-free controls, despite the chlorine demand of fulvic acid.

R. rubra was tested in solutions of fulvic acid. Test results did not deviate significantly from baseline. No disinfection in 50-min contact time was found at pH 9.

TABLE 4. COMPARATIVE FAC 99.99% END POINTS FOR ENTEROVIRUSES AND f_2 COLIPHAGE AT pH 5, 7, AND 9 AT 22°C AND 6°C

Virus	FAC (mg/L)	pH 5 (min)	pH 7 (min)	pH 9	
				Borate (min)	P-C (min)
		22°C			
f_2 Coliphage	1.00	<0.12 (7 sec)	2.0	4.3	--[a]
	0.50	<0.12	3.8	11.3	--
	0.10	3.3	27.5	>50.0	--
Poliovirus I (LSc)	1.00	0.5	0.5	2.3	--
	0.50	2.5	1.5	10.0	--
	0.10	20.0	9.5	41.0	--
		6°C			
f_2 Coliphage	1.00	0.15	2.5	7.0	5.5
	0.50	0.25	5.8	16.0	11.5
	0.25	0.50	11.5	28.0	--
Poliovirus I (LSc)	1.00	2.8	3.8	11.5	9.0
	0.50	7.5	6.5	27.0	16.5
	0.25	11.3	12.8	>0.3	--
	0.10	73.0	32.3	>50.0	--
Poliovirus I (Brunhilde)	1.00	8.0	8.0	17.5	13.7
	0.50	15.3	13.0	36.0	28.1
Coxsackie B3 Virus	3.50	1.0	1.50	18.5	7.0
	2.00	1.30	1.80	26.5	--
	1.00	4.5	5.0	55.0	19.0
	0.50	6.5	7.5	88.0	--
Echovirus 7	1.00	--	--	5.0	--
	0.50	--	--	11.5	--
	0.25	--	--	21.0	--

a. No data.

TABLE 5. FAC DISINFECTION OF E. COLI 11229 IN BUFFERED WATER WITH FULVIC ACID (5 C.U.) AT pH 9 AND 6°C

Amperometric FAC Values (mg/L) Initial	Final	99.99% Inactivation Time	Relationship[a]
Prereacted 50 min			
1.90	1.30	30.0 sec	--
1.00	0.75	50.0 sec	2.4X faster
0.50	0.30	2.3 min	1.4X faster
No Prereaction			
1.00	0.60	1.3 min	1.5X faster
0.50	0.32	5.0 min	1.5X slower
0.20	0.20	5.8 min	--
Cl Demand-Free Controls			
1.00	0.98	2.0 min	
0.50	0.45	3.3 min	
0.25	0.23	12.0 min	

a. Relationship of test times to matching Cl demand-free controls.

Table 6 presents the results of the FAC disinfection of f_2 coliphage in fulvic acid at pH 5, 7, and 9. Prereacted solutions yielded inactivation times similar to chlorine demand-free controls when the FAC levels in the controls were matched with the final FAC concentrations in the test beakers. Non-prereacted test solutions required twice the time to inactivate f_2 at pH 7 and 9 for matching FAC doses in tests and controls.

TABLE 6. FAC DISINFECTION OF f_2 COLIPHAGE IN BUFFERED WATER WITH FULVIC ACID (5 C.U.) AT pH 5, 7, AND 9 AND 6°C

Amperometric FAC Values (mg/L)		99.99% Inactivation Time		
Initial	Final	pH 5	pH 7	pH 9
Prereacted 50 min				
1.55	1.00	<7.0 sec	2.5 min	3.5 min
1.00	0.55	16.0 sec	6.0 min	6.3 min
0.80	0.45	1.8 min	ND[a]	36.0 min
No Prereaction				
1.00	0.75	23.0 sec	3.5 min	6.3 min
0.50	0.40	37.0 sec	6.5 min	16.0 min
0.25	0.20	1.8 min	13.0 min	28.1 min
Cl Demand-Free Controls				
1.00	1.00	<7.0 sec	2.6 min	6.0 min
0.50	0.48	15.0 sec	6.0 min	16.0 min
0.25	0.25	45.0 sec	11.5 min	37.5 min

a. Not done.

Table 7 shows the results of FAC disinfection of poliovirus I (LSc) in 5 C.U. fulvic acid at pH 5, 7, and 9 and 6°C. Inactivation times are given for prereacted, non-prereacted, and chlorine demand-free test solutions. Initial and final amperometric chlorine concentrations are shown. Spot checks of chlorine levels at t=0 (when virus was added) showed that most of the chlorine consumed by fulvic acid had been reacted by the time virus was added. Data from pH 5 studies indicated that the 99.99 percent inactivation times in those test solutions were predictable for each t=0 FAC dose on the basis of chlorine demand-free test results. The exception was for a FAC dose of 0.25 mg/L in the non-prereacted test; the chlorine demand-free control required 14.5 min to inactivate 99.99 percent of the virus while the test solution required more than 50 min. Results at pH 7 and 9 are similar to those at pH 5, including the 0.25 mg/L FAC exception. Again, inactivation times for poliovirus I (LSc) at pH 5 were slower (1.2 to 1.6 times slower) or equal to those at pH 7.

TABLE 7. FAC DISINFECTION OF POLIOVIRUS I (LSc) IN BUFFERED WATER WITH FULVIC ACID (5 C.U.) AT pH 5, 7, AND 9 AND 6°C

Amperometric FAC Values (mg/L)		99.99% Inactivation Time (min)		
Initial	Final	pH 5	pH 7	pH 9
Prereacted 50 min				
1.55	0.80	3.4	3.0	12.0
1.00	0.55	6.0	6.0	24.0
0.80	0.35	ND[a]	15.0	28.0
No Prereaction				
1.00	0.62	3.9	2.7	9.0
0.50	0.32	8.9	5.5	34.0
0.25	<0.20	50.0	>50.0	>50.0
Cl Demand-Free Controls				
1.00	1.00	3.5	3.3	9.0
0.50	0.45	8.4	5.5	25.0
0.25	0.23	14.5	15.0	40.0

a. Not done.

It is interesting to note that the chlorine demand of 5 C.U. fulvic acid does not greatly affect disinfection kinetics of the test organisms except with low (<0.25 mg/L) FAC concentrations. Apparently, chlorine reacts with the microorganisms faster than with the fulvic acid; when sufficient chlorine to satisfy a 30 min chlorine demand is added, many of the naturally shorter inactivation times are reduced even further, as if there were no chlorine demand. When extended disinfection end points are found in chlorine demanding test solutions, then it is conjectured that FAC reacts with the fulvic acid first and the microorganisms second, thus effecting longer end points than in matching chlorine demand-free controls.

Turbidity

Solutions containing 5 NTU bentonite clay with and without 250 mg/L Ca^{+2} were tested for chlorine demand. No chlorine demand was found. *E. coli* 11229 was tested at pH 5, 7, and 9 at 6°C. Results are shown in Table 8, and test results for *R. rubra* are presented in Table 9. Inactivation times for *E. coli* were unaltered from baseline at any pH level, while inactivation times for *R. rubra* showed no change from baseline at pH 5 and 9. The slight increase (30.5%) at pH 7 is probably due to experimental error. It is unlikely the indicated increase was significant.

TABLE 8. FAC DISINFECTION OF E. COLI 11229 IN WATER CONTAINING 5 NTU BENTONITE WITH AND WITHOUT 250 mg/L Ca^{+2} AT pH 5, 7, AND 9 AND 6°C

pH	FAC (mg/L)	99.99% Inactivation Time	
		T[a]	T + Ca^{+2}
5	0.25	<7 sec (0)[b]	<7 sec (0)
7	0.25	30 sec (0)	30 sec (0)
9	0.50	4.1 min (0)	3.9 min (0)

a. T = test water with 5 NTU bentonite turbidity.
b. Values in parentheses indicate percent decrease in inactivation time from Cl demand-free controls.

TABLE 9. FAC DISINFECTION OF R. RUBRA IN WATER CONTAINING 5 NTU BENTONITE WITH AND WITHOUT 250 mg/L Ca^{+2} AT pH 5, 7, AND 9 AND 6°C

pH	FAC (mg/L)	99.99% Inactivation Time	
		T[a]	T + Ca^{+2}
5	2.00	22.5 min (0)[b]	20.0 min (+11.1%)
7	2.00	41.0 min (+30.5%)	41.0 min (+30.5%)
9	5.00	>50.0 min (0)	>50.0 min (0)

a. T = test water with 5 NTU bentonite turbidity.
b. Values in parentheses indicate percent increase in inactivation time from Cl demand-free controls.

Table 10 shows the results of f_2 coliphage tested in turbid water with and without Ca^{+2} at pH 5, 7, and 9 and 6°C. No change in inactivation times from baseline was found for pH 5 and 7 test solutions. However, Ca^{+2} influenced the test at pH 9 by increasing the 99.99 percent virus inactivation times of the bentonite-Ca test solutions by 54 to 64 percent. No change from baseline was found in disinfection rates of the test solutions containing only bentonite.

TABLE 10. FAC DISINFECTION OF f_2 COLIPHAGE IN WATER CONTAINING 5 NTU BENTONITE WITH AND WITHOUT 250 mg/L Ca^{+2} AT pH 5, 7, AND 9 AND 6°C

pH	FAC (mg/L)	99.99% Inactivation Time	
		T[a]	T + Ca^{+2}
5	1.00	<7 sec (0)[b]	<7 sec (0)
	0.50	7-15 sec (0)	7-15 sec (0)
7	1.00	1.5 min (0)	2.0 min (0)
9	1.00	7.0 min (0)	2.5 min (+64%)
	0.50	14.0 min (0)	5.0 min (+63%)

a. T = test water with 5 NTU bentonite turbidity.
b. Values in parentheses indicate percent decrease in inactivation time from Cl demand-free controls.

Table 11 shows the results of the FAC disinfection of poliovirus I (LSc) in water containing bentonite with and without Ca^{+2} at pH 5, 7, and 9 at 6°C. Data from all three pH levels reveal no significant change in inactivation times compared to chlorine demand-free controls. Ca^{+2} had little or no effect in the disinfection rates. Bentonite did not protect the virus from FAC. Turbidity levels permitted by EPA Primary Water Standards[10] apparently will not pose a problem in FAC disinfection of potable water. Although bentonite clay particles are reported to protect viruses from FAC inactivation, this must occur only at higher concentrations of clay than those tested. The effect of Ca^{+2} on f_2 disinfection will be further discussed.

Hardness

Borate-buffered test solutions, maintained at pH 5, 7, and 9 and containing 250 mg/L Ca^{+2}, Mg^{+2}, and K^{+}, were found to have no chlorine demand. Table 12 shows the results of testing E. coli 11229 in such synthetic hard water. No changes in inactivation times from baseline were seen in data for pH 5 and 7. However, at pH 9, decreases of 50 to 60 percent were seen for all FAC levels tested. Because of the great sensitivity of E. coli to FAC, no further studies of the effect of hardness on FAC disinfection were performed with this bacterium.

When R. rubra was tested, with and without Ca^{+2}, at pH 5, 7, and 9, no change from baseline occurred at any pH or FAC level.

TABLE 11. FAC DISINFECTION OF POLIOVIRUS I (LSc) IN WATER CONTAINING 5 NTU BENTONITE WITH AND WITHOUT 250 mg/L Ca^{+2} AT pH 5, 7, AND 9 and 6°C

pH	FAC (mg/L)	99.99% Inactivation Time	
		T[a]	T + Ca^{+2}
5	1.00	3.4 min (+8.1)[b]	2.9 min (+21.6)
	0.50	7.2 min (+2.7)	5.8 min (+21.6)
	0.25	11.0 min (+13.4)	12.4 min (2.4)
7	1.00	2.4 min (+7.7)	1.9 min (+26.9)
	0.50	7.3 min (0)	6.8 min (0)
	0.25	13.0 min (+5.8)	N.D.
9	1.00	12.3 min (0)	8.8 min (+16.2)
	0.50	30.0 min (0)	23.3 min (+14.8)
	0.25	36.0 min (+10.7)	36.0 min (+10.7)

a. T = test water with 5 NTU bentonite turbidity.
b. Values in parenthesis indicate percent decrease in inactivation time from Cl demand-free controls.

TABLE 12. FAC DISINFECTION OF E. COLI 11229 IN BUFFERED WATER CONTAINING 250 mg/L Ca^{+2} AT pH 5, 7, AND 9 AND 6°C

pH	FAC (mg/L)	99.99% Inactivation Time	% Decrease[a]
5	0.25	16 sec	0
	0.10	34 sec	0
7	1.00	4.5 sec	0
	0.50	15 sec	0
	0.25	38 sec	0
9	1.00	40 sec	66.7
	0.50	1.1 min	66.7
	0.25	6.0 min	50.0

a. Percent decrease in inactivation time from Cl demand-free controls.

The greatest decrease in inactivation time was shown by f_2 coliphage at pH 9 with the divalent cations, Mg^{+2} and Ca^{+2}. Inactivation times for f_2 coliphage were unaltered from baseline at pH 7 and 5. Table 13 shows results of testing f_2 at pH 9 in borate buffer with 250 mg/L cation. An average of 86 percent reduction in inactivation times, effected by Ca^{+2} and Mg^{+2}, was found for all FAC concentrations tested. A notable finding was the reduction in inactivation times at 0.15 mg/L FAC; the end point in the chlorine demand-free control was 76 min versus 8 min with Ca^{+2} present and 5.5 min in the presence of Mg^{+2}. The monovalent cation, K^{+}, caused no significant decreases in disinfection end points. In the presence of divalent cations, Ca^{+2} or Mg^{+2}, disinfection was faster at pH 9 than at cation-free controls at pH 7 (Fig. 9).

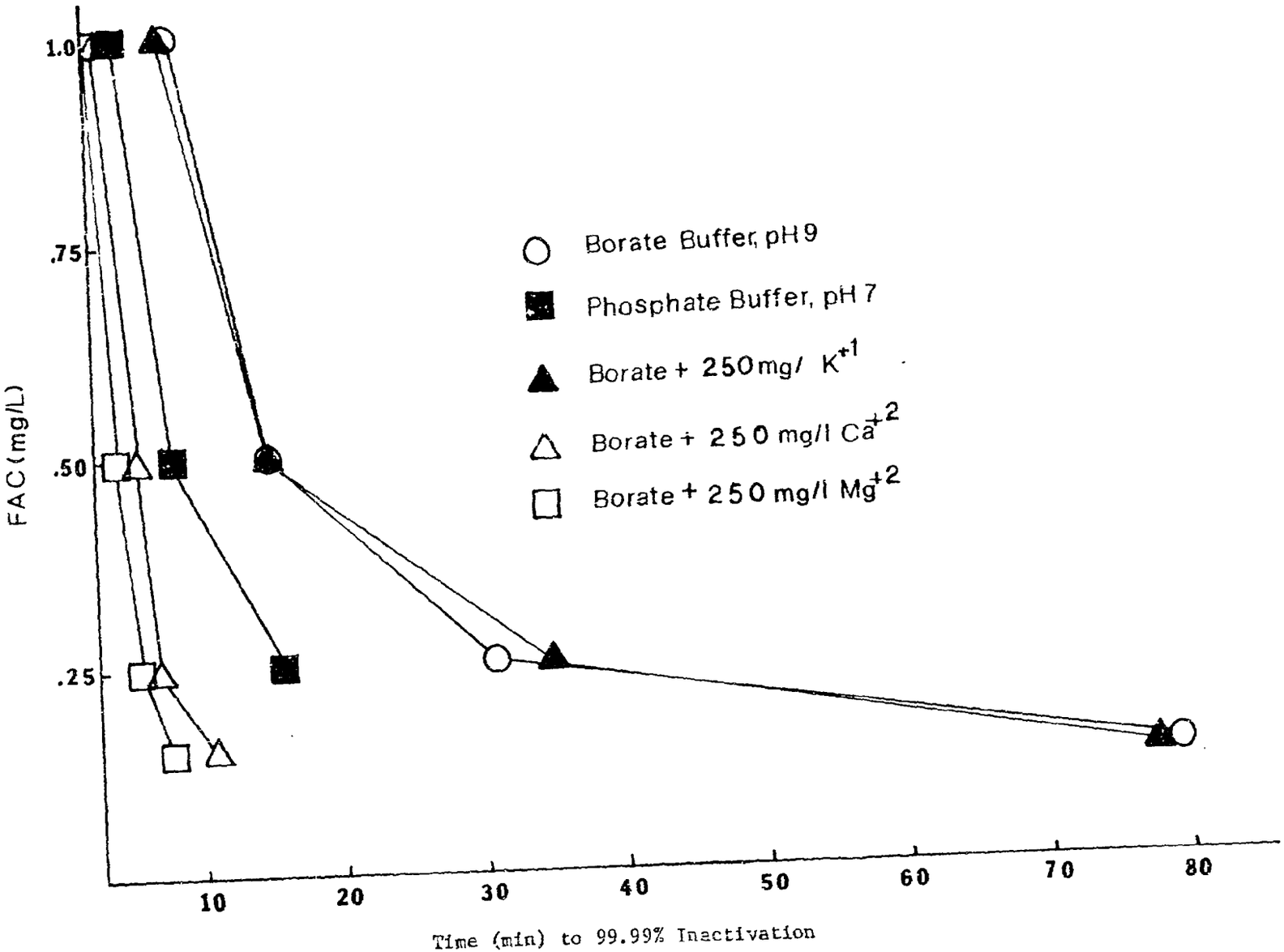

Figure 9. Effects of Cations (Hardness) on FAC Disinfection of f_2 Coliphage at pH 9 and 6°C.

TABLE 13. FAC DISINFECTION OF f_2 COLIPHAGE IN BORATE-BUFFERED WATER WITH 250 mg/L CATION AT pH 9 AND 6°C

FAC (mg/L)	Cation	99.99% Inactivation Time (min)	% Decrease[a]
1.00	Ca^{+2}	1.1	84.3
	Mg^{+2}	1.1	84.3
	K^{+}	5.6	20.0
0.50	Ca^{+2}	2.1	83.3
	Mg^{+2}	2.3	81.8
	K^{+}	12.7	0
0.25	Ca^{+2}	3.1	88.9
	Mg^{+2}	3.8	86.4
	K^{+}	73.0	0
0.15	Ca^{+2}	8.2	89.2
	Mg^{+2}	5.5	92.8
	K^{+}	75.5	0

a. Percent decrease in inactivation time from Cl Demand-Free Controls.

Less dramatic results were seen for poliovirus I (LSc) at pH 9 with divalent cations present (Table 14); no change from baseline values was found at pH 5 and 7. Inactivation time decreases of 30 to 50 percent were effected by the divalent cations, and disinfection still proceeded faster at pH 7 than at pH 9.

Similar experiments were conducted at pH 9 using P-C buffer as test solution with borate buffer solutions serving as chlorine demand-free controls. All controls were run, including P-C, borate, and cation nonchlorinated test solutions. Cations were tested at 0.01 M or 0.05 M ionic strength. FAC levels were 1.0 and 0.50 mg/L for all viruses tested except coxsackie B3, which was tested at FAC levels of 3.5 and 1.0 mg/L. All viruses were tested except echovirus 7, the virus most sensitive to FAC disinfection.

TABLE 14. FAC DISINFECTION OF POLIOVIRUS I (LSc) IN BUFFERED WATER WITH 250 mg/L CATION AT pH 9 AND 6°C

FAC (mg/L)	Cation	99.9% Inactivation Time (min)	% Decrease[a]
1.00	Ca^{+2}	7.8	31.6
	Mg^{+2}	7.8	31.6
	K^{+}	10.6	7.0
0.50	Ca^{+2}	18.9	26.7
	Mg^{+2}	17.8	31.0
	K^{+}	24.0	7.0
0.15	Ca^{+2}	62.5	50.4
	Mg^{+2}	67.5	46.4
	K^{+}	88.0	30.2

a. Percent decrease in inactivation time from Cl demand-free controls.

Table 15 shows the effects of cation and buffer on the disinfection of f_2 virus at pH 9 and 6°C. The data show the greatest decrease in inactivation of f_2 virus was effected by Mg^{+2} or Ca^{+2} in borate buffer. This decrease was not significantly greater when the ionic strength of the test solution was raised to 0.05 M. P-C buffer effected a 23 to 28 percent decrease in f_2 inactivation times; the presence of divalent cations caused a greater decrease of 60 to 83 percent. P-C buffer with divalent cations did not increase the efficacy of FAC disinfection of f_2 at pH 9 as much as did borate buffer with the same cations present, nor were the monovalent cations, K^{+} or Na^{+}, of any significant value.

The effects of buffers and cations on FAC disinfection of poliovirus I (LSc) at pH 9 and 6°C are shown in Table 16. The dose-response of poliovirus I (LSc) to FAC in borate buffer was 20 to 50 percent slower than the response to FAC in P-C buffer. When Ca or Mg was added to the test system, the reaction of poliovirus I (LSc) to FAC increased to 80 to 87 percent over borate buffer. Although the divalent cations exerted the most effective decrease in inactivation time, P-C buffer plus K^{+} effected a 53 percent increase over reactions in borate. Therefore, K^{+} caused an 8 percent decrease in FAC polio I disinfection time over P-C buffer alone. The effect of the increased disinfection rates with cation and P-C buffer is that, in this test system, poliovirus I (LSc) is inactivated as fast at pH 9 as it is at pH 5 and 7, with or without cations present.

TABLE 15. FAC DISINFECTION OF f_2 COLIPHAGE AT pH 9 AND 6°C: EFFECTS OF BUFFERS AND CATIONS

FAC (mg/L)	Buffer[a]	Cation	99.99% Inactivation Time (min)	% Decrease[b]
1.00	B	0.006 M Ca^{+2}	1.1	84.3
	B	0.006 M Mg^{+2}	1.1	84.3
	B	0.006 M K^{+}	5.6	20.0
0.25	B	0.05 M Ca^{+2}	6.0	85.0
		0.05 M Mg^{+2}	6.0	85.0
		0.05 M K^{+}	43.0	0
		0.05 M Na^{+}	48.0	0
1.00	P-C	None	5.4	22.9
0.50	P-C	None	11.5	28.1
1.00	P-C	0.01 M Ca^{+2}	2.6	62.9
		0.01 M Mg^{+2}	1.2	82.9
		0.01 M K^{+}	5.5	21.4
0.50	P-C	0.01 M Ca^{+2}	5.0	68.8
		0.01 M Mg^{+2}	3.3	79.4

a. B = 0.005 M borate.
P-C = 0.003 M phosphate-carbonate.

b. Percent decrease in 99.99% inactivation time from Cl demand-free borate controls.

TABLE 16. FAC DISINFECTION OF POLIOVIRUS I (LSc) AT pH 9 and 6°C: EFFECTS OF BUFFERS AND CATIONS

FAC (mg/L)	Buffer[a]	Cation	99.99% Inactivation Time (min)	% Decrease[b]
1.00	B	0.006 M Ca^{+2}	7.8	31.6
		0.006 M Mg^{+2}	7.8	31.6
		0.006 M K^{+}	10.6	7.0
1.00	P-C	None	9.0	21.1
0.50	P-C	None	18.0	48.6
1.00	P-C	0.01 M Ca^{+2}	4.5	62.7
		0.01 M Mg^{+2}	4.3	62.3
		0.1 M K^{+}	9.0	21.1
1.00	P-C	0.05 M Ca^{+2}	1.5	86.7
		0.05 M Mg^{+2}	2.0	82.3
		0.05 M K^{+}	6.3	44.2
0.50	P-C	0.05 M Ca^{+2}	6.0	82.9
		0.05 M Mg^{+2}	6.2	82.3
		0.05 M K^{+}	16.5	52.9

a. B = 0.005 M borate.
P-C = 0.003 M phosphate-carbonate.

b. Percent decrease in 99.99% inactivation time from Cl demand-free borate controls.

The most dramatic departure from borate baseline disinfection kinetics, effected by P-C buffer, with and without cations present, was found for coxsackie B3 virus. Coxsackie B3 virus in borate buffer was the most resistant of the viruses investigated, requiring more than 2 mg/L FAC at pH 9 and 6°C for 99.99% inactivation within 30 min. Coxsackie B3 was tested with P-C buffer and 0.01 M cations with borate buffer as reference; results are shown in Table 17. The dose-response of coxsackie B3 to FAC in P-C buffer was an average of 64 percent faster than coxsackie B3 in borate. The addition of 0.01 M Ca^{+2} or Mg^{+2} (but not K^{+}) increased the dose-response to 75 percent over borate. Although FAC disinfection of coxsackie B3 virus still proceeded faster at pH 7, results from P-C buffer and cation tests at pH 9 showed that 5 logs of this most FAC resistant virus could be inactivated within 30 min by 1.0 mg/L FAC.

TABLE 17. FAC DISINFECTION OF COXSACKIE B3 VIRUS AT pH 9 AND 6°C: EFFECTS OF BUFFERS AND CATIONS

FAC (mg/L)	Buffer[a]	Cation	99.99% Inactivation Time (min)	% Decrease[b]
3.50	P-C	None	5.0	61.6
1.00	P-C	None	19.0	65.5
3.50	P-C	0.01 M Ca^{+2}	3.8	79.5
		0.01 M Mg^{+2}	4.8	74.1
		0.01 M K^{+}	7.9	57.3
1.00	P-C	0.01 M Ca^{+2}	13.8	74.9
		0.01 M Mg^{+2}	14.8	73.1
3.50	P-C	0.05 M Ca^{+2}	2.0	89.2

a. P-C = 0.003 M phosphate-carbonate.
b. Percent decrease in inactivation time from Cl demand-free borate controls.

The last virus to be tested was poliovirus I (Brunhilde). Only preliminary data were gathered due to time limitations. These data were derived from testing poliovirus I (Brunhilde) against 1.0 mg/L FAC at pH 9 and 6°C in both borate and P-C buffers with 0.01 M and 0.05 M cations present (Table 18). The data indicate faster (22 percent) inactivation times for poliovirus I (Brunhilde) in P-C buffer than in borate buffer. The ionic strength of the test solutions also affects FAC inactivation times for this virus. When 0.05 M Ca^{+2} was added to the borate system, the FAC inactivation time was the same as in P-C buffer without cation; at 0.01 M, Ca^{+2} did not alter the baseline inactivation time. The ionic strength of the divalent cations also affected the FAC disinfection kinetics in P-C buffer. With increasing cation concentrations, there were decreasing inactivation times.

Table 19 compares the P-C buffer-cation inactivation times of poliovirus I (Brunhilde) at pH 9 to the 1.0 mg/L FAC data at pH 7. The 0.05 M cations effected faster inactivation times at pH 9 than at pH 7. In addition, 0.01 M Mg^{+2} gave an essentially equal reaction time at pH 9 as the reaction time at pH 7.

TABLE 18. FAC DISINFECTION OF POLIOVIRUS I (BRUNHILDE) AT pH 9 AND 6°C: EFFECTS OF BUFFERS AND CATIONS

FAC (mg/L)	Buffer[a]	Cations	99.99% Inactivation Time (min)	% Decrease[b]
1.00	B	0.006 M Ca^{+2}	20.5	0
		0.01 M Ca^{+2}	20.0	0
		0.05 M Ca^{+2}	16.0	22.0
1.00	P-C	None	16.0	22.0
1.00	P-C	0.01 M Ca^{+2}	11.5	43.9
		0.05 M Ca^{+2}	5.0	75.6
1.00	P-C	0.01 M Mg^{+2}	9.0	56.1
		0.05 M Mg^{+2}	2.8	86.3
1.00	P-C	0.01 M K^{+}	17.0	17.1

a. B = 0.005 M borate.
P-C = 0.003 M phosphate-carbonate.
b. Percent decrease in 99.99% inactivation time from Cl demand-free controls (borate).

TABLE 19. P-C BUFFER-CATION FAC INACTIVATION TIMES (MIN) FOR POLIOVIRUS I (BRUNHILDE) AT pH 9

99.99% Inactivation Times (min)				
Mg^{+2} (pH 9		Ca^{+2} (pH 9)		FAC pH 7
0.01 M	0.05 M	0.01 M	0.05 M	1.0 mg/L
9	2.8	11.5	5.0	8

Table 20 displays the results of all the viruses tested for the effects of buffer and cations on FAC disinfection at pH 9. Values are mean percentages of the decreases in FAC inactivation time effected by P-C buffer with cations.

TABLE 20. FAC DISINFECTION OF VIRUSES AT pH 9 AND 6°C: MEAN PERCENT INCREASES IN 99.99% INACTIVATION TIMES OVER BORATE CONTROLS EFFECTED BY BUFFERS AND CATIONS

Buffer[a]	Cation	Polio I (LSc)	f_2 Coliphage	Polio I (Brunhilde)	Coxsackie B3
B	250 mg/L Ca^{+2}	30.8	86.04	None	--[b]
	250 mg/L Mg^{+2}	31.5	86.3	None	--
	250 mg/L K^{+}	11.0	5.0	None	--
B	0.01 M Ca^{+2}	63.0	--	20.0	--
	0.01 M Mg^{+2}	62.3	--	--	--
P-C	None	34.5	22.6	22.0	63.6
P-C	0.01 M Ca^{+2}	62.7	64.9	43.9	77.2
	0.01 M Mg^{+2}	62.3	79.9	56.1	73.6
	0.01 M K^{+}	21.1	35.3	17.1	57.3
P-C	0.05 M Ca^{+2}	84.8	--	75.6	89.2
	0.05 M Mg^{+2}	82.3	--	86.3	--
	0.05 M K^{+}	48.6	--	--	--

a. B = 0.005 M borate.
P-C = 0.003 M phosphate-carbonate.
b. No data.

In the borate system, 250 mg/L (0.006 M) divalent cation gave impressive results with f_2 virus, reducing inactivation time in borate alone by 86 percent; for poliovirus I (LSc) inactivation time was reduced by 31 percent. Poliovirus I (Brunhilde) did not exhibit reduced FAC inactivation times with the addition of divalent cation to borate at this concentration; perhaps a greater molarity of cation would produce a reduction. The use of P-C buffer without added cations resulted in reductions of inactivation times from 22.6 percent (f_2 coliphage) to 63.6 percent (Coxsackie B3 virus).

The most significant finding of this study of buffers and cations was the decreased inactivation times for most of the enteroviruses at pH 9. Potable waters with a high pH are usually hard, containing Ca and Mg salts. P-C buffer plus cations resembles natural water more than does borate buffer, and 1.0 mg/L FAC will satisfactorily disinfect a pH 9, cold, test solution containing 7 logs of FAC-resistant virus; if borate were used, more than 2.0 mg/L FAC would be required.

The cause of the cation enhancement of disinfection at pH 9 is essentially unexplored, although some possible hypotheses can be rendered. In this study, all nonchlorinated buffer controls, including those for borate, and P/C, with and without cations, had identical virus titers for a given experimental run, indicating that there was probably little or no aggregation present in any of

them, even though borate has a high aggregative efficiency.[30] This is due to the large dilution effect; the virions simply cannot contact each other. Even a high-titered virus preparation, 10^{11} PFU/mL, will require 1 to 2 hr to aggregate when diluted to 10^{10} PFU/mL.[28] Given a lack of viral aggregation in the nonchlorinated controls, the following sequence of events might explain this cation enhancement:

1. Chlorine is added to virus suspended in P-C and borate buffers of low (0.003 and 0.005 M, respectively) ionic strength.

2. Virus particles in chlorinated borate buffer undergo some aggregation, perhaps due to a loss or reduction in the ionic double layer surrounding the virus. Since most viruses are negatively charged at pH 9, there might be repulsor forces preventing the rapid inactivation of possibly small virus clumps.

3. Virus particles remain dispersed in P-C buffer,[31] which makes the possibility of chlorine-specific aggregation in borate more probable. P-C buffer has an even lower ionic strength than borate, and undoubtedly, with OCl^- present, the same repulsor forces are in operation. Whatever the cause, viruses in P-C buffer are inactivated faster than they are in borate.

4. When divalent cation is present in either the borate or P-C buffer system, FAC inactivation proceeds rapidly. Floyd and Sharp[32] have suggested that polioviruses and reoviruses possess receptor sites for divalent cations. For many virus infections, Ca^{+2} is required for adsorption to the host cell.[26] Given that divalent cation binds to the virus particle, the net electrical charge will switch from negative to positive.[32] The effect of the charge switch on the virus to net positive suggests OCl^- will no longer encounter repulsor forces, but will be attracted to the cation-virus complex, possibly creating an ion pair, effecting the observed rapid disinfection. The amount of divalent cation required to cause this switch is probably directly related to the extent of virus electronegativity, which, in turn, is directly related to the isoelectric point of the virus. Workers in the field of virus concentration have observed that Ca^{+2} or Mg^{+2} uptake by viruses increases with rising cation concentrations. Eventually a saturation or stabilization is reached when further uptake of cation by the virus does not occur.[33,34] Data in this report indicate a saturation level for f_2 coliphage and poliovirus I of ≤250 mg/L for Ca^{+2} and Mg^{+2}. Disinfection rates for these viruses were not increased by 0.01 M or 0.05 M cations. However, coxsackie B3 and poliovirus I (Brunhilde) did show increased rates with increasing levels of cation.

Although the monovalent cation, K^+, had little or no effect on viral inactivation times at the concentration levels used (up to 0.05 M), higher concentrations probably would be efficacious. Sharp et al.[31] have found that 0.1 M NaCl increases the disinfection rate of poliovirus by OCl^- at pH 10 over that of HOCl at pH 6. Scarpino et al.,[16] who reported OCl^- a better disinfectant than HOCl, used KCl in the test medium. The data presented in this study and data presented by others[16,31] strongly suggest that OCl^- is, at least, equally capable of disinfection as HOCl, not only of viruses but of certain bacteria. Since the microorganisms in this study show no rate increases due to cation at pH 5 and 7 (Ca^{+2} binds to microorganisms regardless of pH)[32] the one important fact at issue with FAC disinfection at high pH is the condition--or electrical state--of microorganisms. In most natural fresh

waters there are cations present, predominantly Ca^{+2} and Mg^{+2}. Most lakes and countless wells produce hard water, and great efforts have been exerted in removing these ions. The results of this study indicate water hardness may not be such a bad idea, particularly where water disinfection is concerned.

SYNTHETIC COMPOSITE INSTALLATION WATER

The only compound tested that exhibited chlorine demand in the interference study was fulvic acid. Since composite test water was formulated to be a composite of the compounds used in the individual interference studies, FAC levels chosen for use were those of the fulvic acid study. Because earlier results suggested that prereacted test solutions did not enhance disinfection rates, composite test water solutions were not prereacted with FAC. Instead, FAC was added to test solutions at t=0 after microorganism addition. Figure 10 illustrates the chlorine demand of the composite test water. The initial "added" FAC and FAC measurements at 1 and 30 min are shown. By 1 min there was a rapid drop in FAC followed by a more gradual decrease. As more chlorine is added, more chlorine is consumed, and the main consumption of chlorine occurs 1 min after addition.

Bacteria

All bacteria tested were easily inactivated by FAC levels of from 1.9 to 0.2 mg/L. Table 21 shows the results of experiments with the five bacterial strains in composite test water at pH 9 and 6°C. Chlorine demand-free controls were included in every experiment, and the 99.99 percent inactivation times are shown for the bacteria in both test water and controls. *E. coli* 11229 and enteropathogenic *E. coli* 23985 were the most resistant, and the other strains followed in the same order as in the baseline studies. The data indicated that the bacteria were inactivated as if there were no chlorine demand. Both *E. coli* strains were inactivated an average of 44 percent faster than the chlorine demand-free controls. The right-hand column of Table 21 shows the relationship of the test results to the controls. If a decrease in inactivation times occurred, the appropriate percentage is shown in parentheses to the right. Percentage reductions shown for the *E. coli* strains are nearly the same as those shown by *E. coli* 11229 in the Ca^{+2} hardness experiments (Table 12). *S. boydii*, *S. typhimurium*, and *V. cholerae* showed small or no decreases in disinfection times from the controls. These results indicate that all bacterial strains tested are sensitive to 0.2 mg/L FAC (99.99% inactivation within 30 min) under worst-case conditions of installation water.

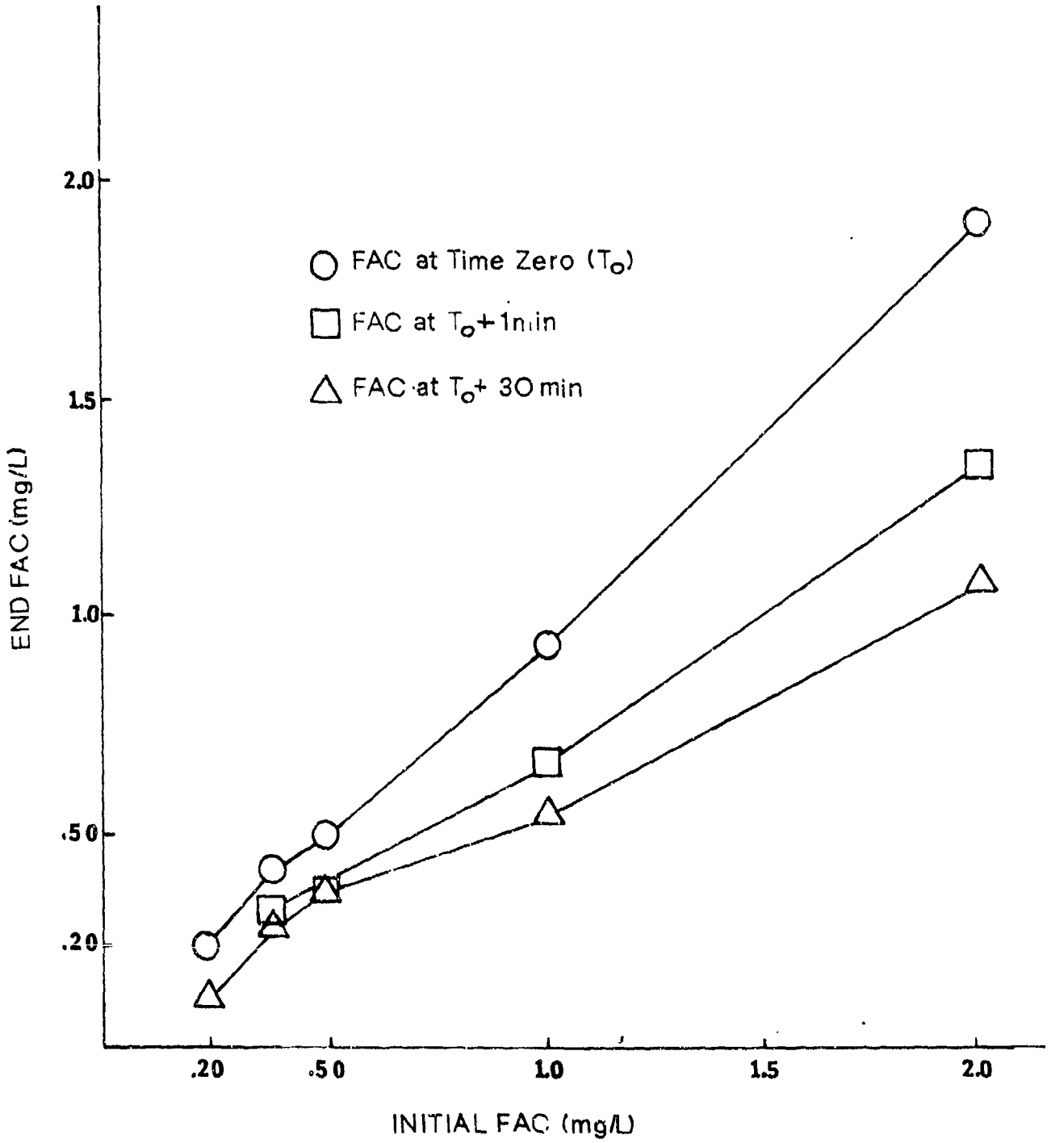

Figure 10. Chlorine Consumption by Synthetic Composite Test Water at pH 9 and 6°C.

TABLE 21. FAC DISINFECTION OF ENTEROBACTERIA IN COMPOSITE TEST WATER AT pH 9 AND 6°C

Microorganism	Initial FAC (mg/L)	99.99% Inactivation Time: Test Water	99.99% Inactivation Time: Cl Demand-Free Controls	Relationship[a]
E. coli 11229	1.9	24.0 sec	ND[b]	-
	1.0	52.0 sec	2.0 min	2.3X faster (56.5)
	0.50	2.5 min	3.3 min	1.3X faster (24.2)
	0.38	3.8 min	ND	-
	0.20	5.8 min	15.8 min	2.7X faster (63.3)
E. coli 23985	1.9	24.0 sec	ND	-
	1.0	41.0 sec	1.2 min	1.8X faster (43.3)
	0.50	1.7 min	2.5 min	1.5X faster (32.0)
	0.38	2.6 min	ND	-
	0.20	5.2 min	4.9 min	1.1 x slower
S. boydii	1.9	11.0 sec	ND	-
	1.0	24.0 sec	30.0 sec	1.3X faster (20.0)
	0.50	1.0 min	1.3 min	1.3X faster (23.0)
	0.38	1.3 min	ND	-
	0.20	3.3 min	3.4 min	0
S. typhimurium	1.9	7.5 sec	ND	-
	1.0	13.0 sec	13.5 sec	0
	0.50	28.0 sec	33.0 sec	1.2X faster (15.0)
	0.38	1.0 min	ND	-
	0.20	2.4 min	2.3 min	0
V. cholerae	1.9	7.0 sec	ND	-
	1.0	10.1 sec	6.0 sec	1.7 x slower
	0.50	19.5 sec	18.0 sec	1.1 x slower
	0.38	42.0 sec	ND	-
	0.20	1.7 min	1.1 min	1.6 x slower

a. Relationship of test times to matching Cl demand-free controls.
b. No data.

Viruses

As in the baseline studies, all the viruses were found to be more resistant to FAC disinfection in composite test water than the Enterobacteriaceae. Table 22 shows the results of testing viruses in composite test water, pH 9 and 6°C. As in Table 20, data derived from test water experiments are listed and compared with data from chlorine demand-free, pH 9 baseline experiments. Data from test water experiments were expected to exhibit slower inactivation times than those from the baseline experiments. However, with the exception of echovirus 7, the viruses were inactivated in the test water in the same time as in chlorine demand-free controls. Coxsackie B3 virus showed an

Initial decrease of 45.7 percent, which was reduced to an average of 25 percent with lower FAC dosages. Poliovirus I (LSc), which showed inactivation time decreases in synthetic hard water, showed none for composite test water; neither did poliovirus I (Brunhilde). Echovirus 7 was the only virus to show the expected increases in inactivation time.

TABLE 22. FAC DISINFECTION OF ENTEROVIRUSES AND f_2 COLIPHAGE IN COMPOSITE TEST INSTALLATION WATER AT pH 9 AND 6°C

Virus	Initial FAC (mg/L)	99.99% Inactivation Time: Test Water	99.99% Inactivation Time: Cl Demand-Free Controls	% Decrease in Inactivation Time in Test Water[a]
f_2 Coliphage	1.9	0.6 min	2.1 min	71.4
	1.0	1.2 min	4.7 min	74.5
	0.50	4.0 min	16.0 min	75.0
	0.38	6.3 min	ND[b]	-
	0.20	17.5 min	65.0 min	73.1
Coxsackie B3	5.00	6.5 min	ND	-
	4.00	7.8 min	ND	-
	3.50	10.0 min	18.4 min	45.7
	2.00	20.5 min	26.5 min	22.6
	1.00	38.0 min	52.7 min	27.9
Poliovirus I (LSc)	1.9	4.5 min	2.8 min	1.6 x slower
	1.0	12.3 min	10.5 min	1.2 x slower
	0.50	21.0 min	27.0 min	22.0
	0.38	29.0 min	ND	-
	0.20	>50.0 min	>50.0 min	-
Poliovirus I (Brunhilde)	1.9	7.0 min	7.8 min	10.3
	1.0	12.8 min	17.5 min	26.9
	0.50	34.0 min	36.0 min	5.6
	0.38	63.0 min	ND	-
	0.20	2.9 hr	ND	-
Echovirus 7	1.9	4.3 min	ND	-
	1.0	6.9 min	5.3 min	1.3 x slower
	0.50	22.8 min	8.8 min	2.6 x slower
	0.20	28.0 min	28.5 min	1.3 x slower

a. If test times were slower than controls, the table indicates as shown.
b. No data.

ENTEROVIRUS DISINFECTION AT pH 5 AND 6°C

During baseline studies it was noted that the disinfection kinetic curves for poliovirus I (LSc), poliovirus I (Brunhilde), and coxsackie B3 virus showed anomalous periods of chlorine resistance when tested at 22°C and particularly at 6°C. Such a period of chlorine resistance was not observed with the bacteria, yeasts, or f_2 coliphage. This resistance was found at FAC levels of 2 mg/L and less. Figure 11 is a set of FAC inactivation curves for poliovirus I (LSc) at pH 5 and 6°C; poliovirus I (Brunhilde) and coxsackie B3 FAC inactivation curves at pH 5 and 6°C were similar. In every instance where this phenomenon occurred, there was an initial rapid rate of disinfection with a loss of approximately 1 to 2 logs virus for poliovirus I (LSc) and 0.8 to 1.0 logs virus for poliovirus I (Brunhilde) and coxsackie B3. Following this initial rapid disinfection, the log percent surviving virus temporarily stabilized (at 2 mg/L FAC) or actually increased in titer (at lower FAC levels). The length of this period of stabilization or resistance to disinfection was inversely proportional to the FAC dose. This lag period was invariably followed by a second linear decrease in virus survival. As a consequence of this anomalous resistance, the time required for 99.99% inactivation of these enteroviruses at pH 5 was significantly lengthened. Thus, contrary to commonly held beliefs of disinfection which postulate that FAC disinfection always proceeds faster at pH 5, poliovirus I (LSc) is inactivated faster at pH 7 than at pH 5, poliovirus I (Brunhilde) is inactivated at the same rate at pH 7 as at pH 5, while coxsackie B3 virus is inactivated only slightly faster at pH 5 than at pH 7.

Although initial and final FAC measurements were the same for a test beaker, an experiment was set up to check for FAC depletion. A test solution containing 0.55 mg/L FAC at 6°C and pH 5 was prepared. After the test beaker was inoculated with poliovirus I (LSc), frequent sampling was done over 180 sec, a time interval known to be well into the second linear inactivation slope. At this point the beaker was reinoculated with a second, identical virus dose. Figure 12 illustrates the results. Amperometric FAC readings before, during, and after the test indicated 0.55 mg/L FAC. Responses of the two virus inoculations were essentially the same: there was an initial rapid drop in virus titer, followed by approximately 2 min of lag and a second rapid drop in virus titer past detection limits (>99.99% decrease).

At this point in the research, it was difficult to explain the inactivation phenomenon. Nonchlorinated pH 5 and 7 controls were aggregate-free, as shown by sucrose gradient analyses. Moreover, other investigators had reported that viral aggregation would not spontaneously occur when dispersed stocks were diluted more than 1:100.[28] Our aggregate-free stock was diluted 1:400 for the test. Similar phenomena were reported at pH 5 or 6 for other enteroviruses.[23,35-37] Finally, Floyd and Sharp[32] found that 0.25 M $MgCl_2$ was effective in preventing acetate-induced aggregation at low pH. In 1980 Jensen et al.[38] reported that 0.1 M NaCl reversed aggregation of coxsackie B5 at pH 6 but not coxsackie B3. With these facts in mind, an experiment was set up using 0.50 mg/L FAC at pH 5 and 6°C. Test beakers contained, at pH 5, 0.25 M $MgCl_2$ plus FAC and 0.005 M acetate buffer plus FAC. The test viruses were those showing the anomalous curve, polioviruses (LSc and Brunhilde) and coxsackie B3. Results are shown in Figures 13, 14, and 15. The effect of $MgCl_2$ was remarkable; disinfection times were reduced and the disinfection kinetic curves became linear. The data suggest that, although these viruses do

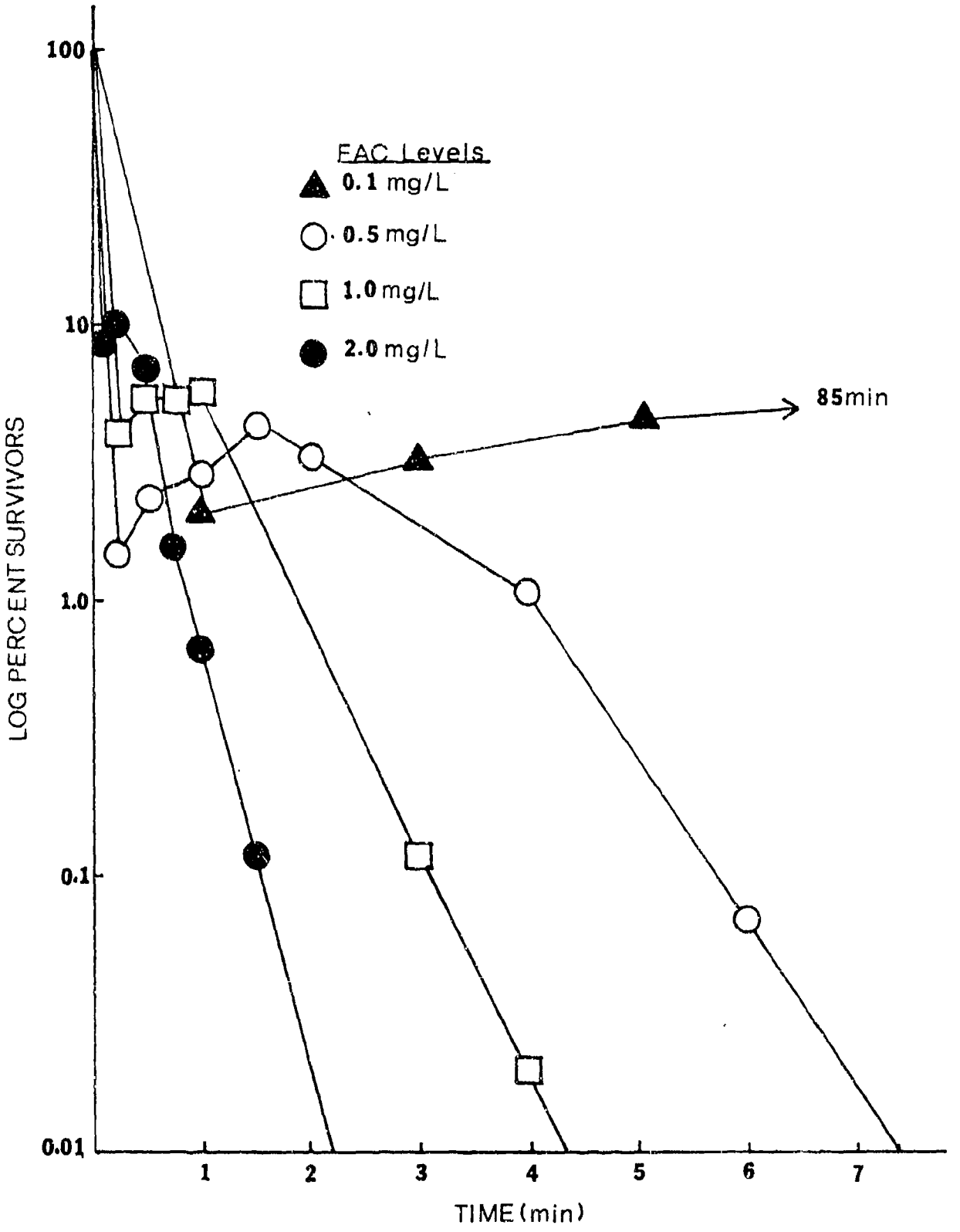

Figure 11. Poliovirus I (LSc): FAC Disinfection at pH 5 and 6°C.

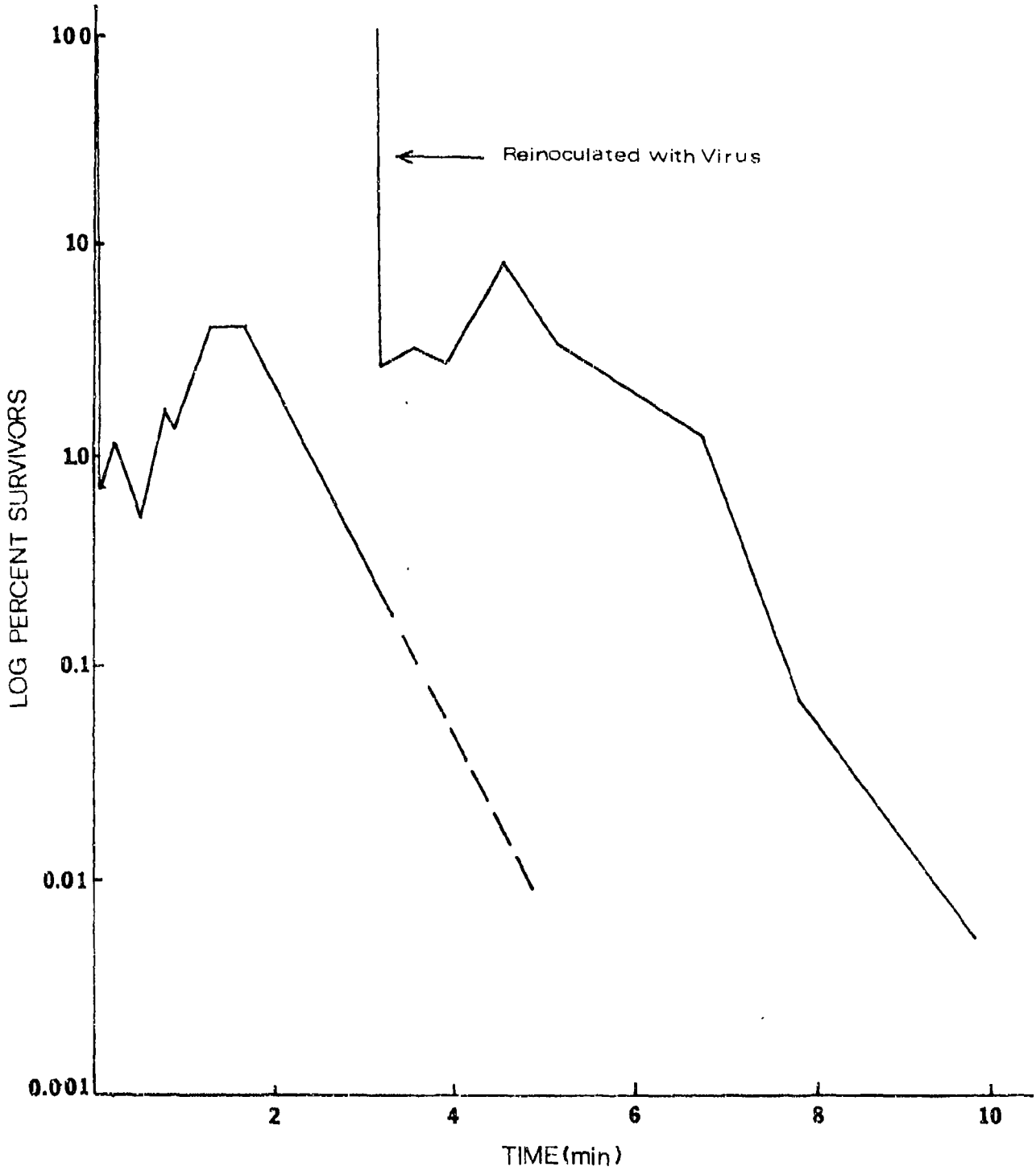

Figure 12. Poliovirus I (LSc): 0.50 mg/L FAC, pH 5 and 6°C.

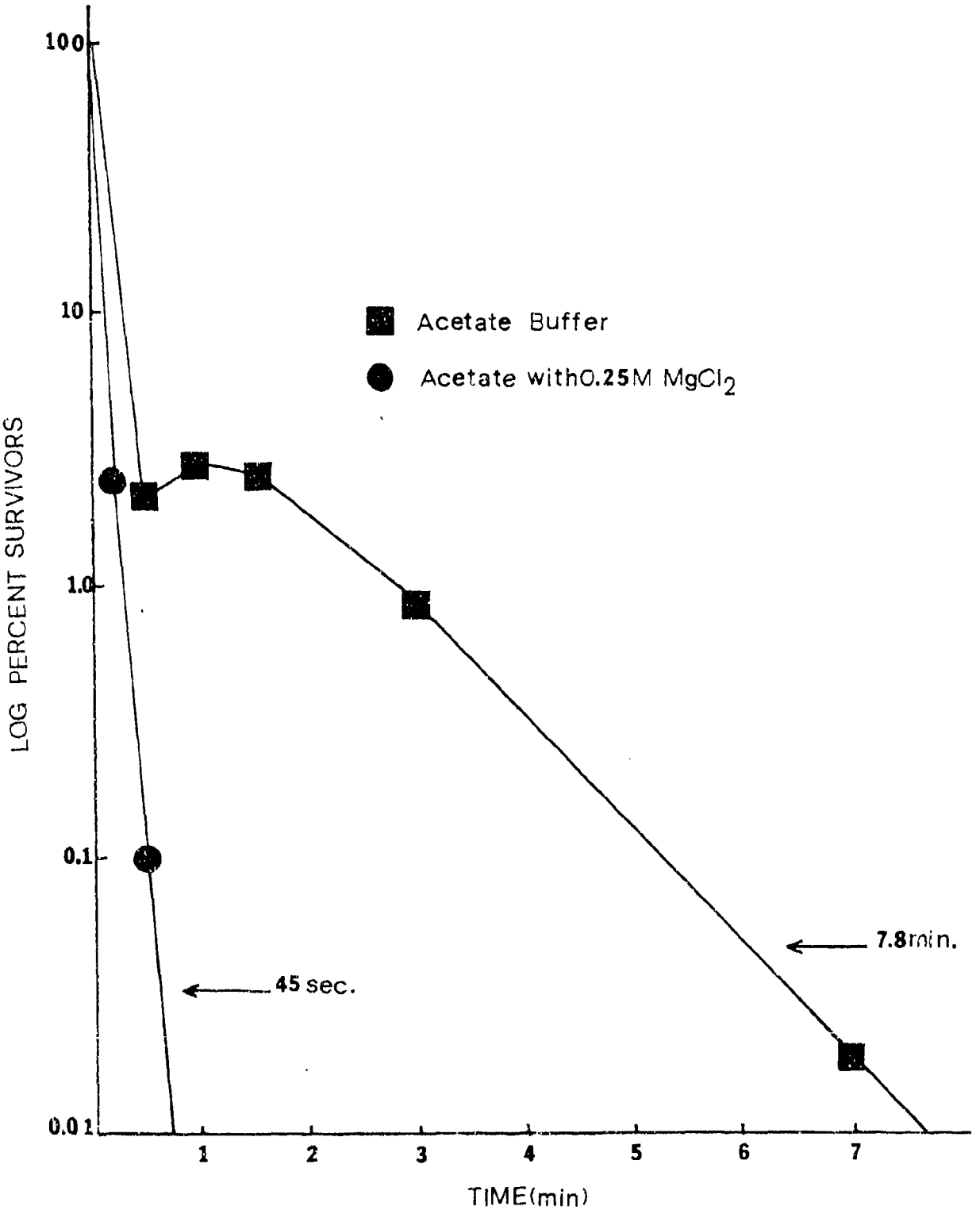

Figure 13. Effect of 0.25 M $MgCl_2$ on Poliovirus I (LSc) at 0.50 mg/L FAC, pH 4.3, and 6°C.

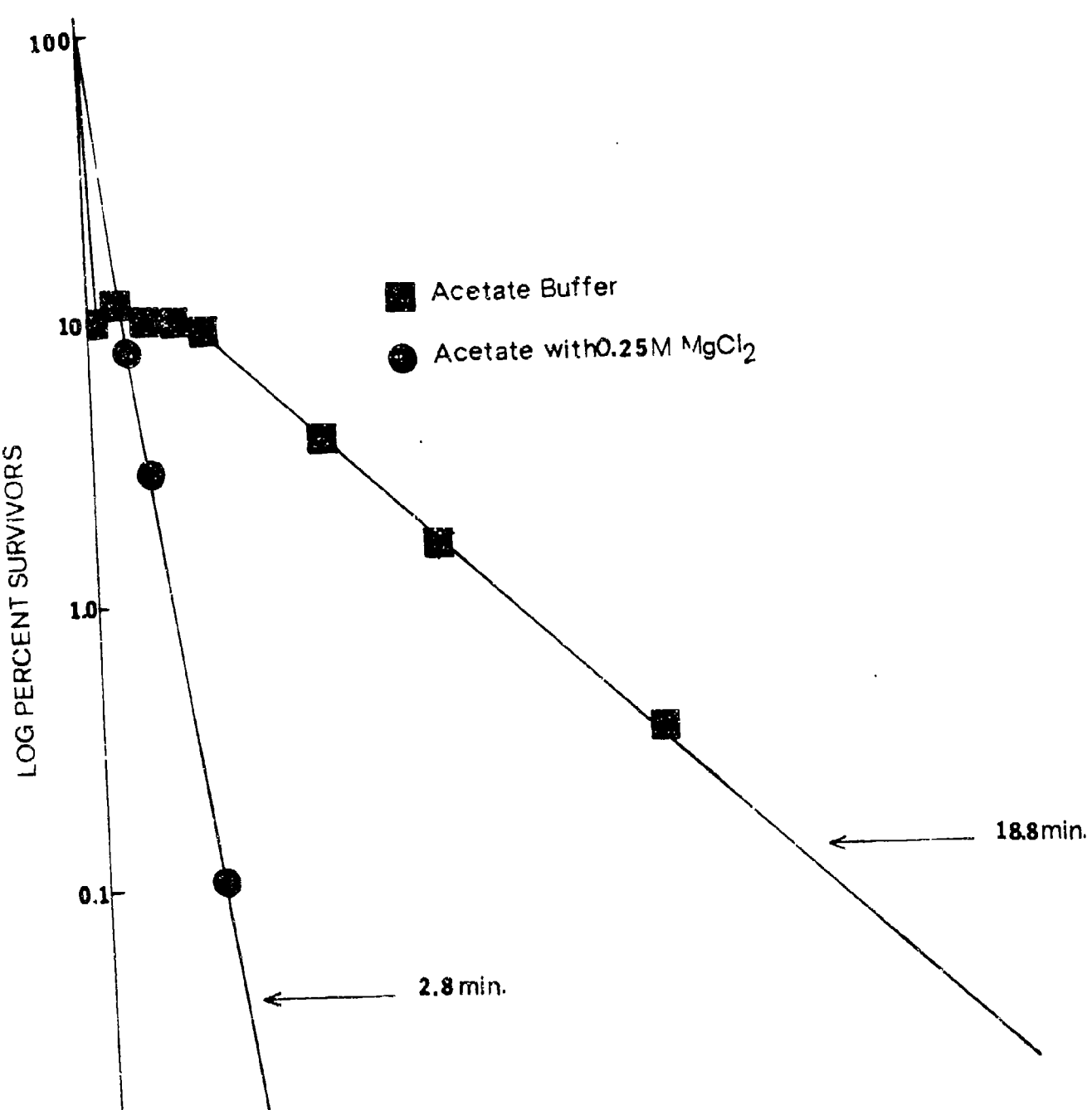

Figure 14. Effect of 0.25 M $MgCl_2$ on Poliovirus I (Brunhilde) at 0.50 mg/L FAC, pH 4.3, and 6°C.

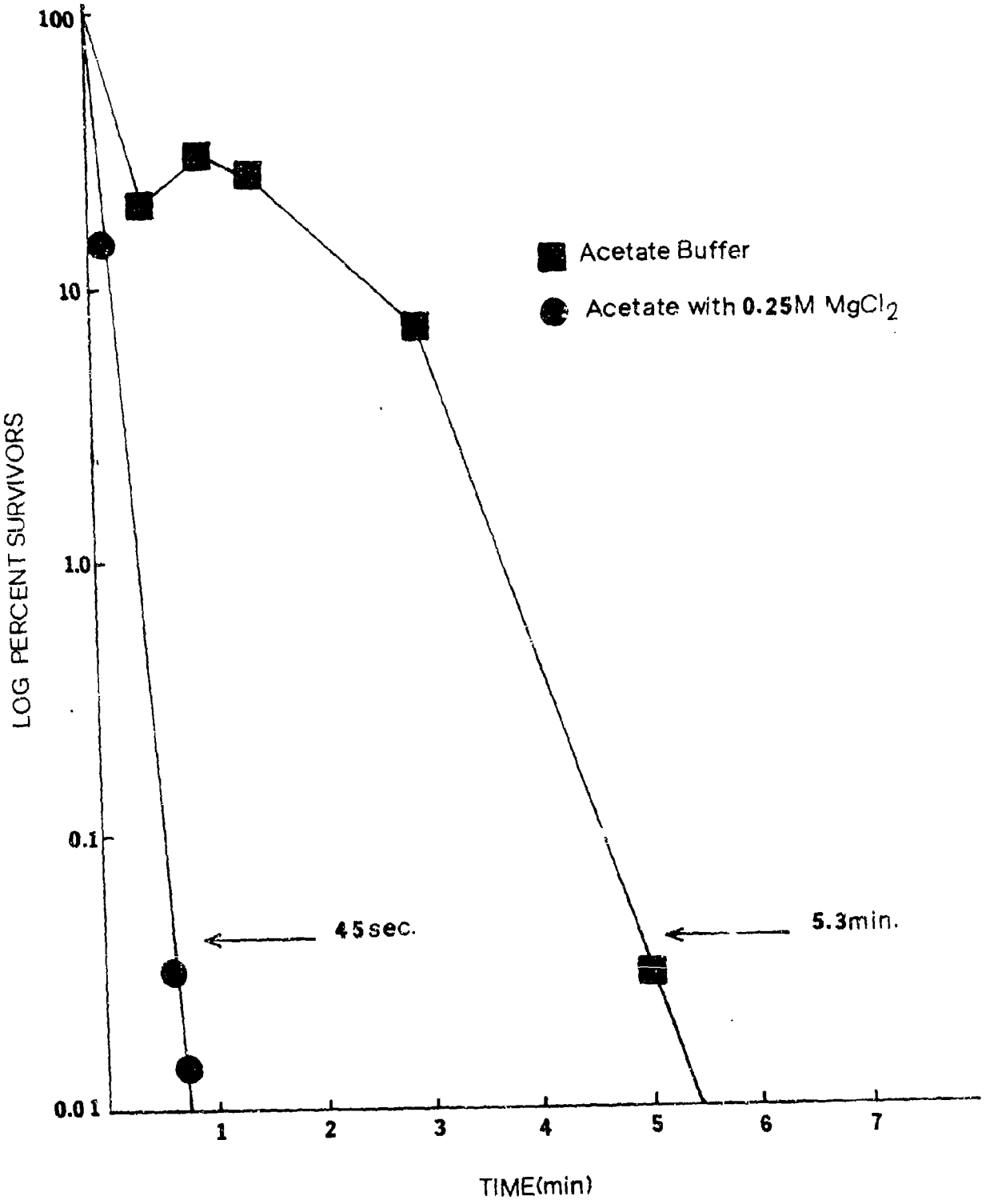

Figure 15. Effect of 0.25 M $MgCl_2$ on Coxsackie B3 Virus at 0.50 mg/L FAC, pH 4.3, and 6°C.

aggregate at low pH when present in high numbers, they do not aggregate when inoculated into the pH 5 nonchlorinated controls. However, in the chlorinated beaker without $MgCl_2$, the viruses underwent chlorine-specific aggregation. Although physically far apart, the viruses seem to be made "sticky" by chlorine; $MgCl_2$ may prevent this by expanding the "ionic double layer" (Floyd and Sharp)[28] probably, as in borate at pH 9, by a virus-cation bridge. The following sequence might describe enterovirus disinfection at pH 5.

1. Viruses are added to 0.005 M acetate at 1:400. Little or no aggregation occurs due to large dilution effect.

2. Viruses aggregate spontaneously as chlorine is added. Any single virions are inactivated in seconds, shown by the first linear decrease in virus survivors on the kinetics curve.

3. Chlorine attacks aggregated virus, yielding degraded "pieces." Neutralization of the sample by sodium thiosulfate in phosphate-buffered saline (PBS) disaggregates clumps. As a result, virus titer in tissue culture increases or stabilizes. Young and Sharp[1] have shown that PBS, the common diluent of virus samples for cell culture, will disaggregate clumped virus.

4. Chlorine eventually erodes the aggregates, momentarily yielding only single virions, which are inactivated as shown by the second linear decrease. The result of linearizing the low pH curves is that the true, monodisperse, baseline end points can be determined. The presence of 0.25 M $MgCl_2$ increased the FAC disinfection rate of the three viruses tested by 7 to 10 times, thereby restoring the pH 5 values (and the known efficacy of HOCl) to what was expected, as shown in Table 23. Values shown are those from acetate (with $MgCl_2$), phosphate and P-C buffers at pH 5,7, and 9, respectively.

TABLE 23. DISINFECTION OF ENTEROVIRUSES BY 0.50 mg/L FAC AT pH 5, 7, AND 9 AND 6°C

Virus	6°C, True Monodispersed Virus 99.99% End Points (min)		
	pH 5	pH 7	pH 9
Poliovirus I (LSc)	0.75	6.5	16.5
Poliovirus I (Brunhilde)	2.8	13.0	28.1
Coxsackie B3	0.75	7.5	prob 30

CONCLUSION

Satisfactory disinfection of potable water supplies at Army installations is dependent upon the following parameters: temperature, pH, ionic strength, turbidity, organic content (chlorine demand), FAC dose, contact time, and type and number of contaminating microorganisms.

TEMPERATURE

In general, higher water temperatures reduce the FAC requirements.

pH

In the past, low pH values were thought to produce faster FAC disinfection than higher pH values. The data presented here show that, although this rule-of-thumb may be true for inactivating bacteria, it is not necessarily true for viruses. All enteroviruses tested showed an anomalous retardation of disinfection at pH 5; other investigators have reported the phenomenon for other viruses.[36] The net result is that many enteroviruses are inactivated as fast or faster at pH 7 as at pH 5. In this study, although the true FAC end points for the dispersed viruses at pH 5 were found, they do not reflect the natural state of viruses in water. In nature, enteroviruses are excreted from the host in large aggregates and usually remain in this state.[1] Preliminary data from this laboratory have shown that enteroviruses subjected to FAC disinfection in naturally acidic field waters exhibit the same anomalous retardation seen in baseline studies.[39]

Alternatively, FAC disinfection of enteroviruses at pH 9 may not be the problem it has always appeared. Ionic strength of the source water determines the disinfection kinetics. The presence of divalent cations decreased the time of FAC end points for enteroviruses and f_2 coliphage by 20 to 90 percent, depending on the virus and the cation concentration. "Soft" water has always been considered highly desirable; hard waters cause scale formation, kill sudsing action, and may even cause a purgative effect.[40] However, if FAC disinfection, especially of hard, alkaline waters, is to be most efficient, then water softening procedures should be undertaken after disinfection.

TURBIDITY AND CHLORINE DEMAND

The turbidity and organic levels used in this study exerted negligible effect on FAC disinfection at any pH tested for both bacteria and viruses. The chlorine demand created by the 5 C.U. fulvic acid exerted a retardation of FAC disinfection rates only with low FAC dosages (<0.50 mg/L) and resistant microorganisms.

MICROORGANISMS

If fecal bacterial species are found in a water supply, then it is likely enteroviruses are also present. There are many cases annually of unidentified gastrointestinal illness that may very well be viral in origin. The data show that the pathogenic viruses tested, poliovirus I (Brunhilde), coxsackie B3, and echovirus 7, are equally, or more resistant to FAC disinfection at the baseline level than the commonly-tested vaccine strain of poliovirus I. Each

viral strain responded differently, which points out the need for determining the disinfection kinetics of all waterborne virus strains.

CONTACT TIME, FAC DOSE, AND DISINFECTION CRITERIA

Potable water supplies less than pH 9 and warmer than 6°C may be safely disinfected by a FAC dose of 1 mg/L for a minimum contact time of 30 min. For cold waters (≤6°C) a FAC dose of 2 mg/L is recommended. These recommended dosages are capable of inactivating coxsackie B3 virus at pH 9 in composite test water; because few source waters have such a high pH, there is a built-in safety factor. If contact times longer than 30 min are available, FAC dosages may be correspondingly reduced.

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