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INACTIVATION OF HEPATITIS A VIRUS AND MODEL VIRUSES IN WATER BY FREE CHLORINE AND MONOCHLORAMINE

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ABSTRACT

The kinetics and extent of inactivation of hepatitis A virus (HAV) as well as three other viruses, coxsackievirus B5 (CB5) and coliphages MS2 and ΦX174, by 0.5 mg/l free chlorine, pH 6-10, and 10 mg/l monochloramine, pH 8, at 5°C in 0.01 M phosphate buffer were determined. HAV was relatively sensitive to 0.5 mg/l free chlorine but relatively resistant to 10 mg/l monochloramine. Compared to HAV, CB5 was quite resistant to inactivation by free chlorine but similar in resistance to inactivation by monochloramine. Inactivation of ΦX174 by free chlorine was rapid at pH 6-9 and intermediate between that of HAV and CB5 at pH 10. ΦX174 was inactivated most rapidly of all viruses tested by 10 mg/l monochloramine. Inactivation of MS2 by free chlorine was somewhat more rapid than HAV at low pH but less rapid than HAV at high pH. MS2 inactivation by 10 mg/l monochloramine was slowest of all viruses tested. These results indicate that HAV is inactivated relatively rapidly by free chlorine but relatively slowly by monochloramine. Coliphage MS2 is a reasonable model to predict inactivation of HAV by free chlorine and inactivation of HAV and CB5 by monochloramine. It is poor model for predicting free chlorine inactivation of CB5 and perhaps some other human enteric viruses.

KEY WORDS

Hepatitis A virus, coxsackievirus, enteroviruses, coliphages, disinfection, inactivation, free chlorine, monochloramine, water.

INTRODUCTION

Hepatitis A virus (HAV) is an important waterborne enteric virus that has caused outbreaks of hepatitis A due to consumption of treated and untreated drinking water in the United States (Lippy and Waltrip, 1984) and elsewhere. In some reported outbreaks the drinking water was chlorinated and met coliform bacteria and other quality standards (Hejkal et al., 1982). The growing number of reports on the isolation of viruses, including HAV, from treated drinking water (Bitton et al., 1986) suggests that some viruses may survive treatment under certain conditions. Establishing reliable water treatment practices and water quality standards to insure the virological safety of water supplies requires knowledge of the response of HAV and other waterborne viruses to disinfection. However, there have been few reports on HAV inactivation by chlorine. Early studies by Neefe et al. (1945, 1947) suggested that HAV is relatively resistant to chlorine. Total and free chlorine concentrations of 1.1 and 0.4 mg/l, respectively, in purified effluent were needed to prevent infectious hepatitis in volunteers. More recently, Peterson et al. (1983) reported that the marmoset infectivity of a partially purified preparation of HAV containing about 1500 infectious units/ml was only partially reduced by treatment with up to 1.5 mg/l of free residual chlorine at neutral pH for 30 minutes. These results, along with observations from the outbreak of hepatitis in Georgetown, Texas (Hejkal et al., 1982), suggest that HAV is more resistant to water chlorination processes than other enteroviruses and indicator bacteria.

In contrast to the studies cited above, those by Grabow et al. (1983) indicated that HAV may be more sensitive to free chlorine than suggested by previous studies and epidemiological evidence. Using HAV infectivity assays in cell cultures, Grabow and co-workers found that HAV was quite sensitive to low levels of free chlorine relative to selected indicator viruses and bacteria. However, further studies indicated that HAV was relatively resistant to combined forms of chlorine in tap water and sewage effluent (Grabow et al., 1984).

Considering the limited data and inconsistent findings of previous reports, there is a need for further studies of HAV inactivation by free and combined forms of chlorine under controlled conditions. Such studies are now feasible using new methods for the cultivation and enumeration of HAV in cell cultures (Daemer et al., 1981; Lemon et al., 1983). The purpose of this present study is to determine the kinetics and extent of HAV inactivation in water by free chlorine and combined chlorine in the form of monochloramine at defined pH levels and temperature. Inactivation of HAV is compared to the inactivation of model viruses including coxsackievirus B5 and bacteriophages MS2 and ϕX174.

METHODS AND MATERIALS

Viruses, Cell Cultures and Virus Purification

HAV. The HM175 strain of HAV, originally isolated from feces of an infected human in Australia (Daemer et al., 1981; Gust et al., 1985), is produced in persistently infected BS-C-1 cells grown at 37°C. HAV infectivity is assayed by the radioimmunofocus assay (RIFA) in BS-C-1 cells as previously described (Lemon et al., 1983; Sobsey et al., 1985). Persistently infected cells and culture fluid harvests are centrifuged at ca. 3,000 x g, resuspended in small volumes of phosphate-buffered saline (PBS), pH 7.5, and extracted by homogenizing in an equal volume of chloroform. The HAV-containing PBS is recovered by low speed (5-10,000 x g) centrifugation. The cell debris and chloroform are further extracted four to six more times with equal volumes of PBS and then twice more with equal volumes of 0.1% SDS in PBS to obtain additional virus. All PBS and SDS-PBS extracts are recovered by low speed centrifugation at room temperature or 4°C, and SDS is removed by precipitation and centrifugation at 4°C.

HAV in cell culture fluids is concentrated by precipitation with polyethylene glycol 6000 (PEG) (12% w/v, pH 7.2) overnight at 4°C. Precipitates are recovered by low speed centrifugation, resuspended in a small volume of PBS and extracted with an equal volume of chloroform. PBS extracts are cleared of chloroform and PEG by low speed centrifugation. HAV in pooled PBS extracts of cells and PEG precipitates is pelleted by ultracentrifugation at 105,000 x g for 4 hours at 5°C. HAV pellets are resuspended in small volumes of 0.01M phosphate-buffered, halogen demand-free (PBHDF) water, supplemented with CsCl to give a density of 1.33 g/ml, and ultracentrifuged to equilibrium in self-generated gradients at 90,000 x g and 5°C for 3 days. Peak fractions of HAV from CsCl gradients are desalted by ultrafiltration and washed with PBHDF water using Centricon 30 ultrafiltration tubes (Amicon Inc). Desalted fractions are layered onto 10-30% sucrose gradients in PBHDF water, pH 7.5, and ultracentrifuged in the SW27 rotor (Beckman Instruments) at 90,000 x g and 5°C for 5 hours. Harvested gradient fractions corresponding to single virions are then pooled and mixed with appropriate amounts of single virions of the other test viruses. The titer of each virus in the mixture is about 1-5 x 10^8 infectious units/ml.

Coxsackievirus B5. CB5 (Faulkner strain) is grown and assayed by the plaque technique in BGM cells as previously described (Sobsey et al, 1978). Virus in cell lysates is liberated from cell debris by freezing and thawing, and then centrifuging at low speed for 15-30 minutes. Viruses in resulting supernatants are supplemented to 0.1% SDS and pelleted by ultracentrifugation (105,000 x g and 5°C for 4 hours). Resulting virus pellets are resuspended in PBHDF water, homogenized 1 minute, and centrifuged at 5,000 x g and 5°C for 20 minutes to remove additional debris and precipitated SDS. After supplementing with CsCl to a density of 1.33 g/ml, viruses are banded to equilibrium as for HAV. CsCl gradient fractions containing the virus peak are desalted and subjected to rate-zonal centrifugation in 10% to 30% sucrose gradients as for HAV. Sucrose gradient fractions corresponding to single virions are added to HAV samples to give the desired virus titer.

Bacteriophages. Coliphages MS2 (ATCC 15597-B1) and ϕX174 (ATCC 13706-B1) are grown and assayed by the top agar plaque technique (Adams, 1959) in E. coli C3000 (ATCC 15597) and E. coli C (ATCC 13706) hosts, respectively, using nutrient agar #2. Crude virus is harvested from the top agar of plaque assay plates having confluent lysis by scraping into small volumes (3-5 ml/plate) of PBS. Harvests are extracted with chloroform and centrifuged at 10,000 x g for 10 minutes. Viruses in the resulting supernatant are pelleted by ultracentrifugation for

4 hours at 105,000 x g and 5°C. Pellets are resuspended in PBHDF water, supplemented with CsCl to give a density of 1.44-1.45 g/ml, and the viruses are banded to equilibrium in CsCl gradients for 3 days at 90,000 x g and 5°C. Gradient fractions with the virus peak are desalted using Centricon 30 ultrafilters and then filtered successively through Tween-80-treated, 0.2 and 0.08 μm pore size polycarbonate filters (Nuclepore) to remove virus aggregates. The filtrates, containing almost exclusively single virions, are combined with single virions of HAV and CB5.

Glassware, Chlorine Reagents and Chlorine Analysis

Glassware for disinfection experiments is soaked >4 hours in a 10-50 mg/l free chlorine solution and then rinsed thoroughly with HDF water. HDF water and buffer solutionss are prepared from twice deionized, activated carbon-filtered water which is then passed through a macroreticular scavenging resin bed (Rohm and Haas). HDF, phosphate-based buffers, 0.01 M, are used to prepare test solutions for disinfection experiments. Household bleach (5.25% sodium hypochlorite; Clorox) is diluted in HDF water to prepare a 100 mg/l stock solution. Monochloramine stock solution of about 100 mg/l is prepared after Berman and Hoff (1984) by combining equal volumes of a 200 mg/l free chlorine solution and an 800 mg/l NH_4Cl solution, both in 0.01 M phosphate buffer, pH 9.5. Stock chlorine solutions are then diluted in test water (PBHDF water, pH 6-10) to give the target chlorine concentrations (0.5 mg/l free chlorine and 10 mg/l monochloramine).

Chlorine concentrations are measured by DPD colorimetric methods, and these procedures are standardized by the DPD ferrous titrametric method (American Public Health Association, 1985). Chlorine samples from the U.S. Environmental Protection Agency are analyzed regularly as check samples.

Protocol for Disinfection Experiments and Data Analysis

Test samples are placed in 25 mm dia. x 150 mm long test tubes and kept in a water bath at 5°C. A 0.24 ml volume of purified, monodispersed virus mixture (HAV, CB5, MS2 and ϕX174) is added to 11.76 ml of a chlorine solution containing 0.51 mg/l free chlorine (or 10.2 mg/l monochloramine) and then briefly mixed. A second test tube containing only chlorine solution serves as a halogen control. A third tube containing a 1:50 dilution of stock virus in PBHDF water serves as a virus control. Samples of 0.7 ml are withdrawn from the reaction tube (chlorine solution plus added virus) for viral analysis at 0.33, 1, 3, 10, 30 and 60 minutes after virus addition. These samples are immediately diluted two-fold in 2X Eagle's MEM containing 1% $Na_2S_2O_3$ and stored at 4°C for subsequent virus assays. For virus assay, samples are further diluted serially 10-fold in separate diluents for HAV, CB5 and the two phages. After the 60 minute reaction period, the remaining test mixture (halogen plus added virus) and the chlorine control sample are re-analyzed for free and combined chlorine. Samples from the virus control (virus in PBHDF water) are diluted serially 10-fold at the beginning and the end of the 60 minute reaction period for subsequent virus assays.

Virus disinfection data, as plaque forming units (PFU) per ml for CB5, MS2 and ϕX174 or radioimmunofocus forming units (RFU) per ml for HAV, are average values from triplicate cultures. For each experiment, the virus concentrations of the virus control sample at time = 0 are computed and taken as N_o, the initial virus concentration. Virus concentrations in control samples did not change appreciably over 60 minutes. For each test sample (samples taken from the test mixture at 0.33, 1, 3, 10, 30 and 60 minutes), the average concentration of each virus is computed. The proportion of initial viruses remaining at each test time (t) is computed by dividing the virus concentration at each test time (N_t) by the initial virus concentration (N_o). These values are then $\log_{10}$-transformed ($\log_{10}[N_t/N_o]$), and the values of duplicate or triplicate experiments are averaged. These mean data for $\log_{10} N_t/N_o$ are then paired with the data for sampling time (t) and analyzed by linear regression to obtain the correlation coefficient, slope of the regression line, and estimated time for 99.99% inactivation of the initial viruses.

RESULTS AND DISCUSSION

The mean results of duplicate or triplicate experiments at 5°C in PBHDF water using 0.5 mg/l free chlorine, pH 6 to 10, and 10 mg/l monochloramine, pH 8, are summarized in Table 1 as times for 99.99% inactivation of the initial viruses (T-99.99).

TABLE 1 Inactivation of HAV, CB5 and Coliphages MS2 and ϕX174 at 5°C in 0.01 M Buffer by 0.5 mg/l Free Chlorine at pH 6, 7, 8, 9 and 10 and 10 mg/l Monochloramine at pH 8

Chlorine Form	pH	Time (Min.) for 99.99% Inactivation			
		HAV	CB5	MS2	ϕX174
Free	6.0	6.5	13.2	1.2	0.5
	7.0	3.6	24	4.4	0.4
	8.0	5.6	52.5	16.7	0.8
	9.0	7.7	108	16	4.6
	10.0	49.6	826	26.5	111
Monochloramine	8.0	117	104	420	31.4

Inactivation by Free Chlorine

The mean results of free chlorine experiments at pH 6, 8, and 10 also are given in Figures 1A, 1B and 1C, respectively, where $\log_{10} N_t/N_o$ is plotted versus contact time in minutes. HAV was inactivated rapidly by 0.5 mg/l free chlorine at pH 6 to 9, with T-99.99 values of <8 minutes (Table 1; Figures 1A and 1B). At pH 10, HAV was inactivated quite slowly (T-99.99 = 49 minutes) (Table 1; Figure 1C), indicating considerable resistance to OCl^-. The similarity of HAV inactivation rates from pH 6 to 9 suggests considerable sensitivity to low levels of HOCl.

The rate and extent of HAV inactivation by free chlorine observed here is slower than that reported by Grabow et al. (1983) but greater than that reported by Peterson et al. (1983). At initial free chlorine concentrations of about 0.4 mg/l and 25°C in 0.05 M buffers, Grabow et al. (1983) obtained T-99.99 values of about 0.25-0.3 minutes at pH 6 and 8 and about 1 minute at pH 10. Their more rapid inactivation could be due to a higher temperature (25°C versus 5°C), higher buffer concentration (0.05 M versus 0.01 M), different HAV strain (MBB versus HM175), different virus assay method, or other factors. Disinfection is more rapid at higher temperatures (Hoff, 1986), and differences among virus strains in sensitivity to disinfection have been observed (Shaffer et al., 1980; Payment et al., 1985). Enhanced inactivation by chlorine at higher buffer concentration or ionic strength, especially at higher pH levels, has been attributed to cation pairing with hypochlorite ion (Jensen et al., 1980).

In contrast to HAV, CB5 was relatively resistant to inactivation by free chlorine, with T-99.99 values ranging from a low of 13.6 minutes at pH 6 to a high of 826 minutes at pH 10 (Table 1; Figures 1A-1C). Inactivation of CB5 by free chlorine gave a typical pattern of decreased inactivation rates at higher pH levels, presumably due to greater resistance to inactivation by OCl^- than by HOCl. Our inactivation rates for CB5 are slower than those of Engelbrecht et al. (1980). With about 0.5 mg/l free chlorine at 5°C, Engelbrecht et al. (1980) reported T-99 values of 3.4, 4.6 and 66 minutes at pH 6, 7.8 and 10, respectively. Our data give T-99 values of about 7, 26 and 413 minutes at pH 6, 8 and 10, respectively, which are slower by factors of about two to six. The reasons for this difference are uncertain, but a higher buffer concentration (0.05 M versus 0.01 M) in the study by Engelbrecht et al. (1980) could have enhanced virus inactivation by ion pairing, especially at higher pH levels.

Inactivation of bacteriophage MS2 by free chlorine was most rapid at pH 6 (T-99.99 = 1.2 minutes) and became generally slower at higher pH levels, with a T-99.99 value of 26.5 minutes at pH 10 (Table 1; Figures 1A-1C). MS2 inactivation was faster than HAV at pH 6 and 10, similar to HAV at pH 7, and slower than HAV at pH 8 and 9. MS2 was inactivated more rapidly than CB5 at all pH levels. Bacteriophage ϕX174 was inactivated more rapidly than either HAV or CB5 between pH 6 and 9, with T-99.99 values ranging from only 0.5 minutes at pH 6 to 4.6 minutes at pH 9 (Table 1; Figures 1A and 1B). At pH 10, where OCl^- predominates, ϕX174 inactivation was slower than HAV but faster than CB5 (Table 1; Figure 1C).

Inactivation by Monochloramine

The mean results of duplicate experiments for 10 mg/l monochloramine in PBHDF water at pH 8 and 5°C are shown in Table 1 as times for 99.99% virus inactivation and in Figure 2 where $\log_{10} N_t/N_o$ is plotted versus contact time. Monochloramine inactivation of HAV and CB5 was similar, with T-99.99 values of 117 and 104 minutes, respectively. MS2 was most resistant to monochloramine (T-99.99 = 420 minutes), and ϕX174 was least resistant (T-99.99 = 31.4 minutes). These results are consistent with previous studies showing that monochloramine is a relatively poor virucide (Berman and Hoff, 1984; Hoff, 1986). In addition, our results for

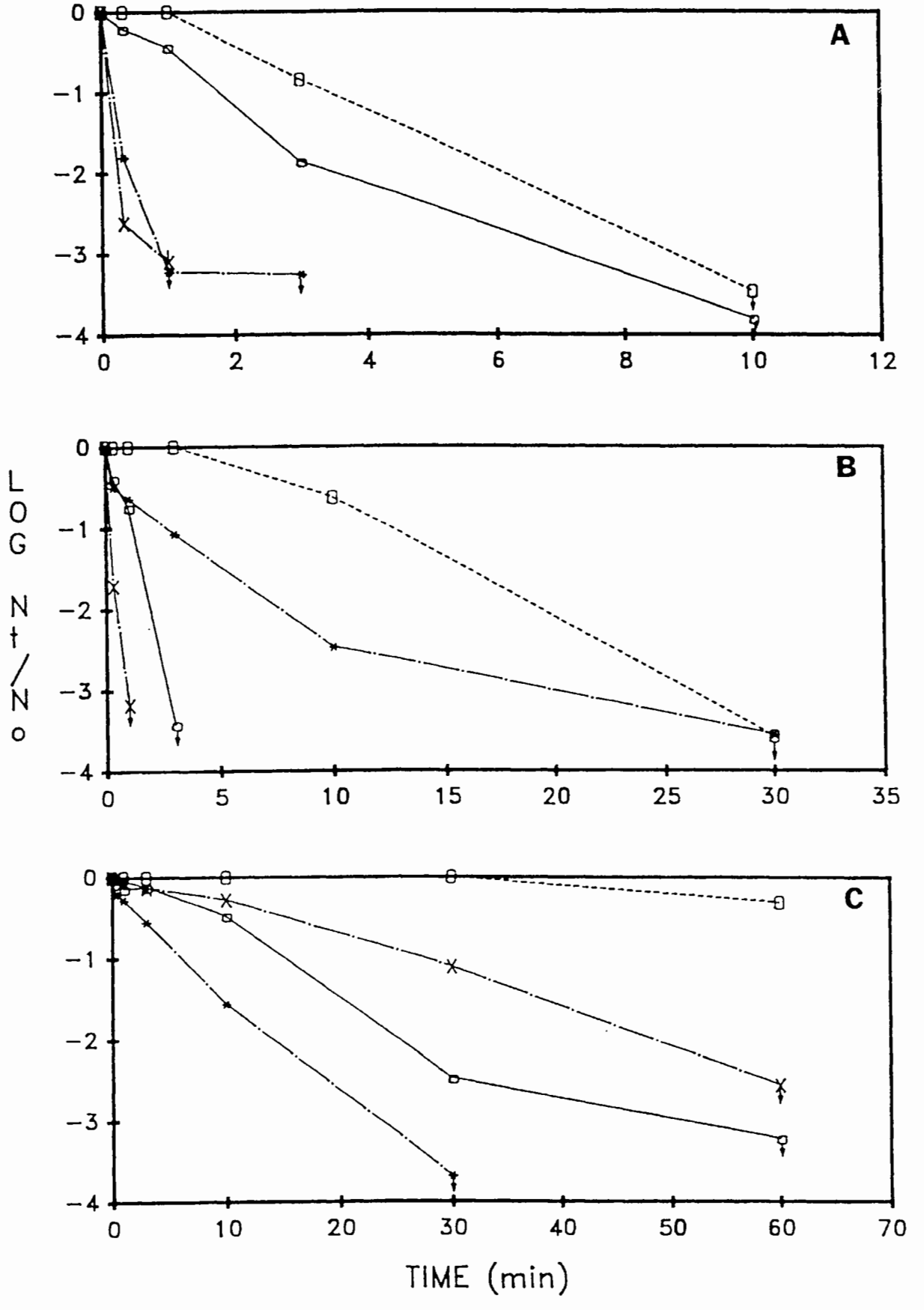

Figure 1. Inactivation of HAV (—□—), CB5 (- - □ - -), MS2 (- —*— -) and OX174 (- —X— -) by 0.5 mg/l Free Chlorine at 5oC and pH 6 (Panel A), pH 8 (Panel B) and pH 10 (Panel C). Arrow (↓) denotes the limit of virus detection.

HAV and MS2 are generally consistent with those of Grabow et al. (1984). In tapwater containing 0.1 mg/l free chlorine and about 11 mg/l chloramines at pH 8, they obtained 99.99% inactivation of HAV in 40 minutes and only 97% inactivation of MS2 in 60 minutes.

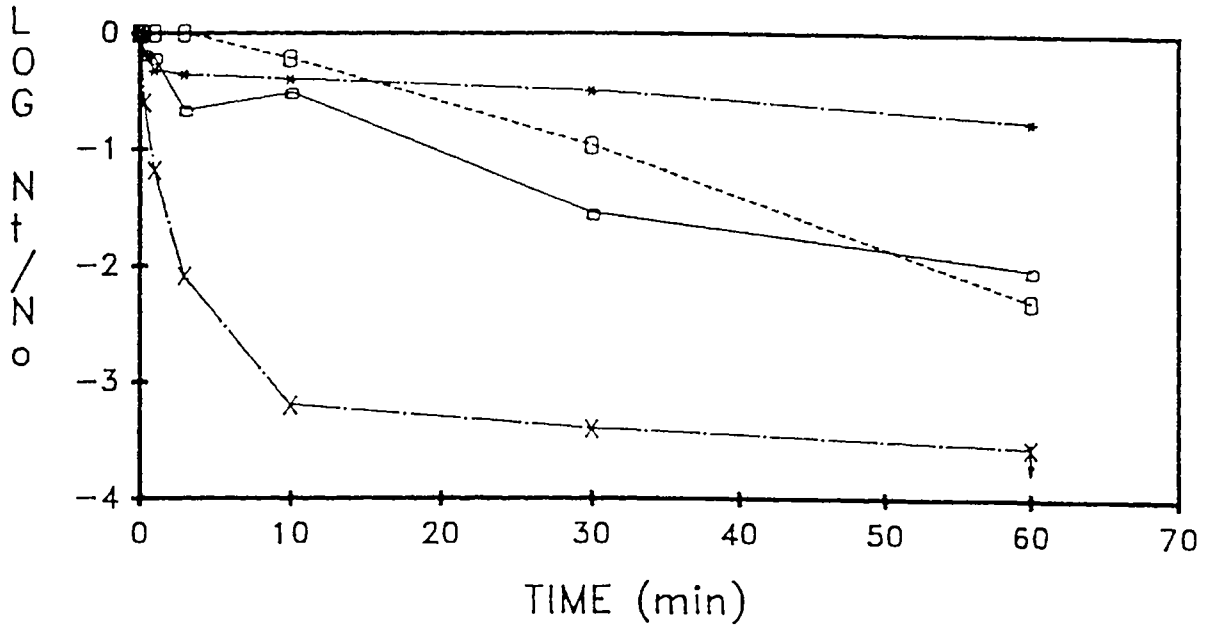

Figure 2. Inactivation of HAV (—▭—), CB5 (··□··), MS2 (·—✱—·) and ΦX174 (·—╳—·) by 10 mg/l Monochloramine at pH 8 and 5°C. Arrow (↓) denotes the limit of virus detection.

Considering that monochloramine was tested at a 20-fold higher concentration than free chlorine, it was a relatively poor virucide. Because monochloramine is typically used in drinking water at only 1-2 mg/l, it appears to be a poor primary disinfectant for HAV and other viruses in drinkling water, unless contact times are extremely long.

Factors Influencing Virus Disinfection

A factor which may influence virus disinfection at different pH levels is the degree of virus aggregation. Aggregation was controlled in stock virus preparations by using only gradient fractions or filtrates containing single particles. However, the addition of monodispersed viruses to reaction mixtures at different pH levels may have caused virus aggregation, thus resulting in slower inactivation kinetics. Previous studies have shown that acid pH levels can induce virus aggregation and decrease inactivation rates (Young and Sharp, 1985).

Another factor which may influence virus inactivation rates at different pH levels is the conformational form of the virus. A form of the virus existing at one pH may be more resistant to disinfection and/or less infectious than another form existing at another pH. Both poliovirus 1 and echovirus 1 can exist in at least two different, pH-dependent conformational forms (Young and Sharp, 1985). Different conformational forms of HAV have not been established, but preliminary evidence from this laboratory suggests possibly two conformational forms of HAV HM175 (unpublished results).

Sensitivity to disinfection sometimes differs among strains of the same virus type. Studies on the disinfection of other strains of HAV by free and combined chlorine are needed in order to determine if the response of strain HM175 is typical or representative of other strains.

SUMMARY

Results of this study indicate that HAV strain HM175 is relatively sensitive to free chlorine and more sensitive than CB5. Coliphages MS2 and ΦX174 also were relatively sensitive to free chlorine, thus making them poor indicators for free chlorine disinfection of enteric viruses

such as CB5. Both phages were more resistant than or similar in resistance to HAV at some pH levels. MS2 was more like HAV than was ϕX174 with respect to inactivation by free chlorine. This suggests that MS2 and similar phages may be useful indicators of HAV disinfection by free chlorine. All four viruses were relatively resistant to monochloramine. HAV and CB5 had similar resistance to monochloramine, while MS2 was most resistant and ϕX174 was most sensitive. These results suggest that MS2 and similar coliphages may be useful indicators of enteric virus disinfection by monochloramine. The considerable resistance of test viruses to monochloramine makes it a poor choice as a primary disinfectant for drinking water.

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REFERENCES

Adams, M.H. (1959) Bacteriophages, Interscience, New York.

American Public Health Association (1985) Section 408, pp. 294-325, Standard Methods for the Examination of Water and Wastewater, 16th ed. American Public Health Association, Washington, D.C.

Berman, D. and J.C. Hoff (1984) Inactivation of simian rotavirus SA11 by chlorine, chlorine dioxide, and monochloramine, Appl. Environ. Microbiol., 48:317-323.

Bitton, G., S.R. Farrah, C.L. Montague and E.W. Akin (1986) Viruses in drinking water, Environ. Sci. Tech., 20:216-222.

Daemer, R.J., S.M. Feinstone, I.D. Gust and R.H. Purcell (1981) Propagation of human hepatitis A virus in African green monkey kidney cell cultures: primary isolation and serial passage, Infect. Immun., 32:388-393.

Engelbrecht, R.S., M.J. Weber, B.L. Salter and C.A. Schmidt (1980) Comparative inactivation of viruses by chlorine, Appl. Environ. Microbiol., 40:249-256.

Grabow, W.O.K., V. Gauss-Muller, O.W. Prozesky and F. Deinhardt (1983) Inactivation of hepatitis A virus and indicator organisms in water by free chlorine residuals, Appl. Environ. Microbiol., 46:619-624.

Grabow, W.O.K., P. Coubrough, C. Hilner and B.W. Bateman (1984) Inactivation of hepatitis A virus, other enteric viruses and indicator organisms in water by chlorine, Wat. Sci. Tech., 17, Amsterdam, 657-664.

Gust, I.D., N.I. Lehmann, S. Crowe, M. McCrone, S.A. Locarnini and C.R. Lucas (1985) The origin of the HM175 strain of hepatitis A virus, J. Infect. Dis., 151:365-367.

Hejkal, T.W., B. Keswick, R.L. LaBelle, C.P. Gerba, Y. Sanchez, G. Dreesman, B. Hafkin and J.L. Melnick (1982) Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis, J. Am. Water Works Assoc., 74:318-321.

Hoff, J.C. (1986) Inactivation of Microbial Agents by Chemical Disinfectants, EPA/600/2-86/067, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Jensen, H., K. Thomas and D.G. Sharp (1980) Inactivation of coxsackieviruses B3 and B5 in water by chlorine, Appl. Environ. Microbiol., 40:633-640.

Lemon, S.M., L.N. Binn and R.H. Marchwicki (1983) Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures, J. Clin. Microbiol., 17:834-839.

Lippy, E.C. and S.C. Waltrip (1984) Waterborne disease outbreaks - 1946-1980: a thirty-five year perspective, J. Am. Water Works Assoc., 76:60-67.

Neefe, J.R., J. Stokes, J.B. Baty and J.G. Reinhold (1945) Disinfection of water containing a causative agent of infectious hepatitis, J.A.M.A., 128:1076.

Neefe, J.R., J.B. Baty, J.G. Reinhold and J. Stokes (1947) Inactivation of the virus of infectious hepatitis in drinking water, Am. J. Pub. Health, 37:365-372.

Payment, P., M. Trembly and M. Trudel (1985) Relative resistance to chlorine of poliovirus and coxsackievirus isolates from environmental sources and drinking water, Appl. Environ. Microbiol., 49:981-983.

Peterson, D.A., T.R. Hurley, J.C. Hoff and L.G. Wolfe (1983) Effect of chlorine treatment on infectivity of hepatitis A virus, Appl. Environ. Microbiol., 45:223-227.

Shaffer, P.T.B., T.G. Metcalf and O.J. Sproul (1980) Chlorine resistance of poliovirus isolates recovered from drinking water, Appl. Environ. Microbiol., 40:1115-1121.

Sobsey, M.D., H.R. Jensen and R.J. Carrick (1978) Improved methods for detecting enteric viruses in oysters, Appl. Environ. Microbiol., 36:121-128.

Sobsey, M.D., S.E. Oglesbee and D.A. Wait (1985) Evaluation of methods for concentrating hepatitis A virus from drinking water, Appl. Environ. Microbiol., 50:1457-1463.

Young, D.C. and D.G. Sharp (1985) Virion conformational forms and the complex inactivation kinetics of echovirus by chlorine in water, Appl. Environ. Microbiol., 49:359-364.

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16. ABSTRACT

The kinetics and extent of inactivation of hepatitis A virus (HAV) as well as three other viruses, coxsackievirus B5 (CB5) and coliphages MS2 and X174, by 0.5 mg/l free chlorine, pH 6-10, and 10 mg/l monochloramine, pH 8, at 5°C in 0.01 M phosphate buffer were determined. HAV was relatively sensitive to 0.5 mg/l free chlorine but relatively resistant to 10 mg/l monochloramine. Compared to HAV, CB5 was quite resistant to inactivation by free chlorine but similar in resistance to inactivation by monochloramine. Inactivation of X174 by free chlorine was rapid at pH 6-9 and intermediate between that of HAV and CB5 at pH 10. X174 was inactivated most rapidly of all viruses tested by 10 mg/l monochloramine. Inactivation of MS2 by free chlorine was somewhat more rapid than HAV at low pH but less rapid than HAV at high pH. MS2 inactivation by 10 mg/l monochloramine was slowest of all viruses tested. These results indicate that HAV is inactivated relatively rapidly by free chlorine but relatively slowly by monochloramine. Coliphage MS2 is a reasonable model to predict inactivation of HAV by free chlorine and inactivation of HAV and CB5 by monochloramine. It is poor model for predicting free chlorine inactivation of CB5 and perhaps some other human enteric viruses.

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