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Potentiation of the Virucidal Effectiveness of Free Chlorine by Substances in Drinking Water

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At 5°C, poliovirus 1 was inactivated by free chlorine (FC) at pH 9.0 more than 10 times as rapidly in drinking water as in purified water. Because ions that comprise many salts potentiate the virucidal effectiveness of FC, we believe that ions and possibly other substances in the drinking water potentiated the virucidal effectiveness of FC. Since viruses may be much more sensitive to chlorination in drinking waters than laboratory tests in purified waters have heretofore led us to believe, it may be possible to reduce the amounts of FC applied to many water supplies for disinfection and thereby perhaps reduce the quantities of halomethanes and other toxic compounds produced in these supplies by the chlorination process.

Certain ions potentiate the virucidal effectiveness of free chlorine (FC). The virucidal effectiveness of FC at pHs 4.5 and 9.0 is potentiated in 0.007 M KCl (K^+, 275 mg/liter; Cl^- 250 mg/liter) (2), and, at a somewhat higher concentration, H_3BO_3-NaOH buffer also potentiates the virucidal effectiveness of FC at pH 9.0 (2). It seems likely that many of the ions present in drinking waters potentiate the virucidal effectiveness of FC.

The study presented herein demonstrates that the virucidal activity of FC at pH 9.0 is markedly potentiated by substances in drinking water.

MATERIALS AND METHODS

Water. Purified water was prepared in a Millipore Super Q system. Drinking water was obtained directly from a well-flushed tap in Cincinnati, Ohio.

Stock chlorine solution. A stock chlorine solution was prepared by bubbling gaseous chlorine into purified water. The stock chlorine solution was stored in the dark until used.

Phosphate buffer. A 0.2 M phosphate buffer solution was prepared by adding 23.7 g of KH_2PO_4 (Fisher P-382) and 4.2 g of K_2HPO_4 (Fisher P-288) to 1 liter of purified water. This solution was filtered through a 0.45-μm membrane (Millipore Corp.). A 0.05 M phosphate buffer solution was prepared by adding 250 ml of 0.2 M phosphate buffer to 750 ml of purified water.

Sodium thiosulfate neutralizer solution. A 100-mg/ml solution of $Na_2S_2O_3$ was prepared by adding 12.5 g of $Na_2S_2O_3$ (Fisher S-446) to 125 ml of purified water. One milliliter of this solution was added to 1 liter of 0.05 M phosphate buffer to yield a 100-mg/liter solution of $Na_2S_2O_3$ in phosphate buffer.

Beef extract (1%). A 1% solution of beef extract was prepared by adding 10 g of beef extract (GIBCO M00040) to 1 liter of purified water. This solution was used for preparing dilutions of virus.

Chlorine-demand-free waters. Chlorine-demand-free waters were prepared by a procedure described in *Standard Methods* (1). Waters, purified and tap, were made demand free by chlorinating them to a free residual of 5 mg/liter and storing them for 3 days. The waters were then dechlorinated by exposure to UV light for several days. To make certain that no chlorine remained in the waters, amperometric titrations were performed on them when chlorine was no longer demonstrable by DPD test. The demand-free dechlorinated waters were stored until needed.

Chlorine-demand-free NaOH. Appropriate-strength solutions of NaOH were prepared and made chlorine demand free. Chlorine was added to each solution of base to a final concentration of 5 mg/liter. The chlorinated solutions of base were stored in the laboratory for 3 days to satisfy the chlorine demand, and the solutions were dechlorinated by exposing them to UV light. To make certain that no chlorine remained, amperometric titrations were performed on the solutions when chlorine was no longer demonstrable by the DPD test.

Chlorine-demand-free glassware. Chlorine-demand-free glassware was prepared by a procedure described in the *USEPA Manual of Methods for Virology* (5) and made chlorine demand free by a procedure described in *Standard Methods* (1). Chlorine-demand-free beakers and cylinders used in the test procedures were rinsed three times in the test waters just before the tests were done.

Amperometric titrations for FC. Amperometric titrations with phenylarsene oxide for FC were done by the procedures described in *Standard Methods* (1). The titrations were done on a total volume of 300 ml of each test fluid.

Virus. A chlorine-demand-free preparation of poliovirus 1 (Mahoney LP, RKP42 of 7 October 1969) was used in all of the experiments described herein.

Virus assays. All poliovirus assays were done in BGM cells (7) by the plaque technique (8). Plaques were generally read 3, 4, and 7 days after cultures were inoculated.

Test procedure. Chlorine-demand-free water, 1.5- to 2-liter quantities of sometimes purified and sometimes tap water, were adjusted to pH 9.0 with 2 and 0.25 M NaOH. About 6 mg of NaOH was required to bring 1 liter of tap water to pH 9.0, and about 2.5 mg of NaOH was required to bring 1 liter of purified water to pH 9.0. Neither quantity of NaOH was sufficient to affect significantly the virucidal effectiveness of FC. Both waters were chlorinated to either 1- or 5-mg/liter residuals, and 400-ml quantities of each were distributed into 600-ml beakers. The pH of each test water was rechecked and readjusted if necessary at the beginning

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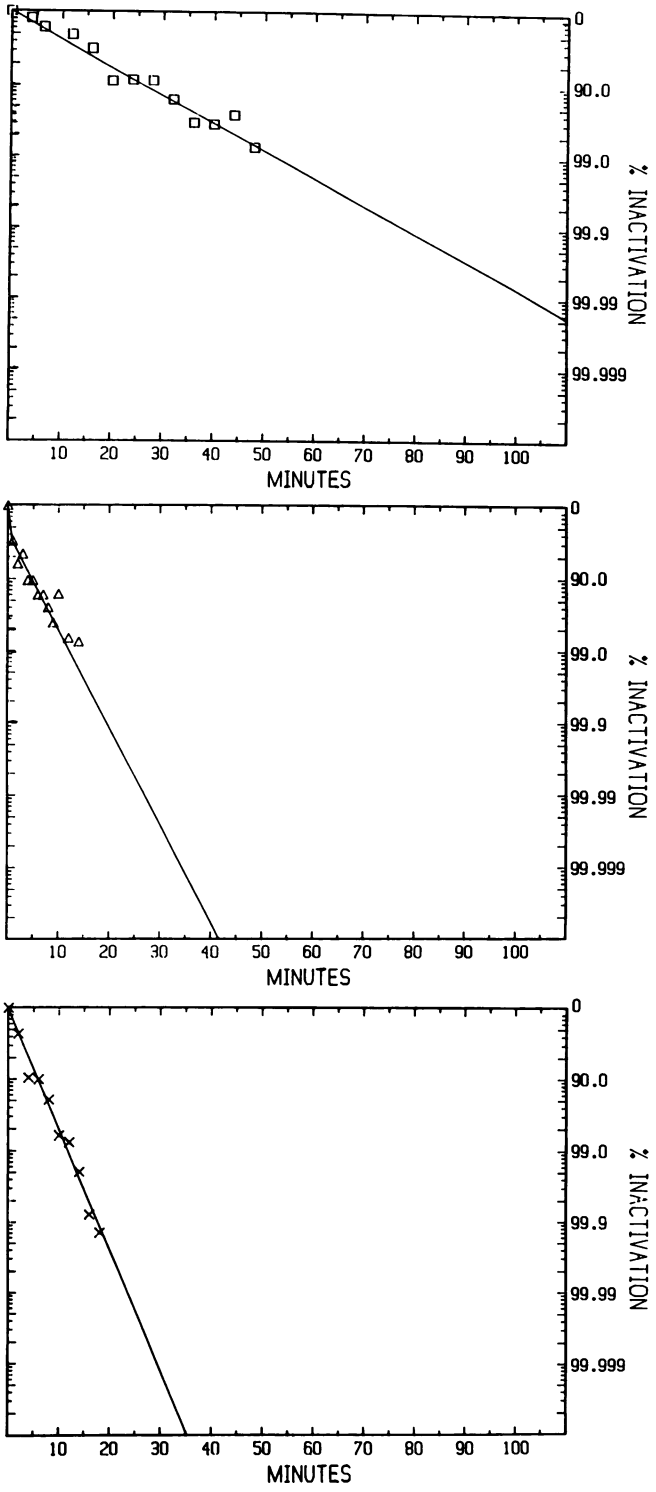

FIG. 1. Inactivation of poliovirus 1 at 5°C by FC at pH 9.0 in purified water. (Top) FC at 0.95 mg/liter. (Center and bottom) FC at 5.00 mg/liter.

of each test. Two control beakers were set up with each test. In most experiments, one beaker contained 400 ml of the test water and the second beaker contained 200 ml of the test water and 200 ml of $Na_2S_2O_3$ neutralizer solution. Test and control waters were brought to 5°C (±1°C) in a water bath, and under continuous stirring on a magnetic stirrer, 1 ml of a chlorine-demand-free poliovirus 1 suspension was added to each beaker. Samples (5 ml) were removed from each test beaker at appropriate time intervals (±1 s) and mixed rapidly with 5-ml volumes of a neutralizer solution that contained 100 mg of $Na_2S_2O_3$ per liter. A similar sampling procedure was used for the control beakers, but the first control beaker was sampled only after 1 min, and the second control beaker was sampled only at the longest time interval used in any of the tests. The pH was determined in each test beaker at the conclusion of each test. Amperometric titrations for FC were also done on all test fluids at the conclusion of each test. All neutralized test samples were diluted in 1% beef extract and inoculated onto cell cultures. Some neutralized test samples were stored at −70°C for several days before dilution and inoculation onto cell cultures. Control tests showed that freezing test samples at −70°C before inoculation onto cell cultures did not alter results.

RESULTS

Inactivation curves were obtained by plotting semilogarithmically (on a percent basis) the fraction of poliovirus 1 inactivated at each sampling time interval.

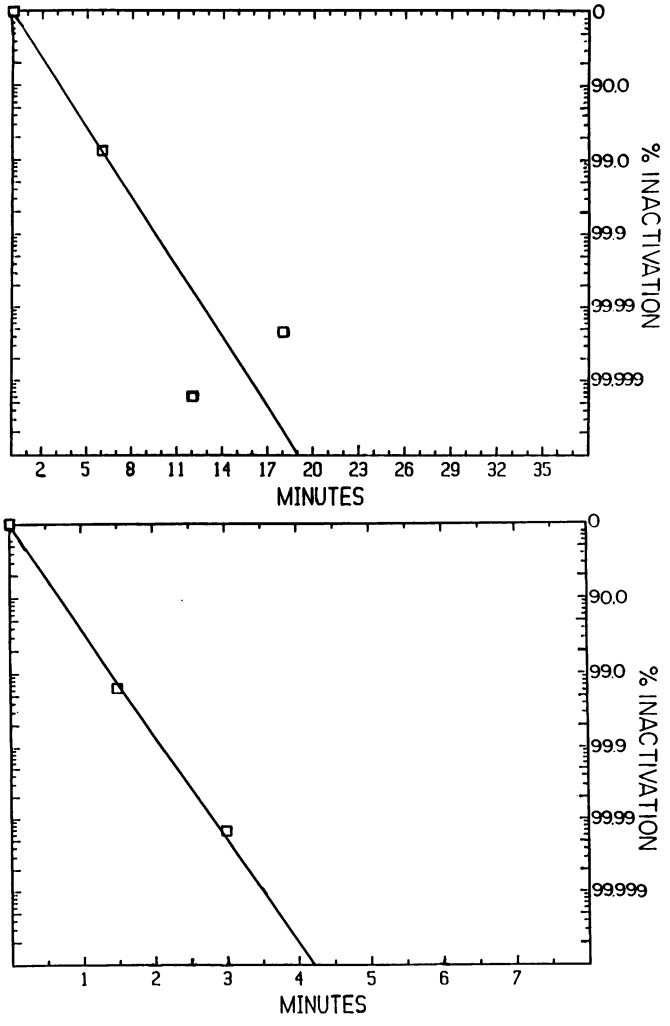

FIG. 2. Inactivation of poliovirus 1 at 5°C by FC at pH 9.0 in Cincinnati drinking water. (Top) FC at 0.95 mg/liter. (Bottom) FC at 5.00 mg/liter.

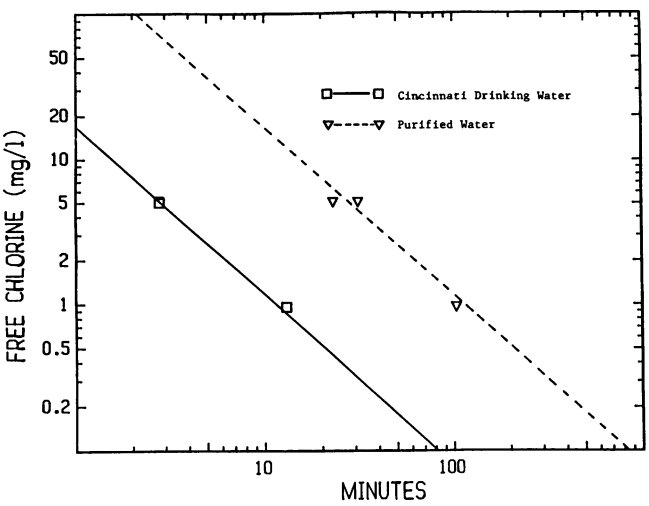

FIG. 3. Relative rates for inactivation of 99.99% of poliovirus 1 at 5°C by FC at pH 9.0 in purified water and in Cincinnati drinking water.

Inactivation of poliovirus 1 in purified water by FC at pH 9.0. In purified water at pH 9.0, 0.95 mg of FC per liter inactivated 99.99% of poliovirus 1 in a little more than 100 min (Fig. 1, top). In the same system, 5.00 mg of FC per liter inactivated 99.99% of poliovirus 1 in 28 to 29 min (Fig. 1, center). In another series of tests in the same test system, 5.00 mg of FC per liter inactivated 99.99% of poliovirus 1 in about 23 min (Fig. 1, bottom).

Inactivation of poliovirus 1 in drinking water by FC at pH 9.0. In drinking water at pH 9.0, 0.95 mg of FC per liter inactivated 99.99% of poliovirus 1 in about 13 min (Fig. 2, top). In the same drinking water system, 5.00 mg of FC per liter inactivated 99.99% of poliovirus 1 in about 3 min (Fig. 2, bottom).

Comparison of inactivation rates for poliovirus 1 in purified and drinking water by FC at pH 9.0. To compare the inactivation rates for poliovirus 1 in purified and drinking water, van't Hoff plots (3, 4) were prepared from the data in the inactivation curves (Fig. 1 and 2). For this purpose, the time required to inactivate 99.99% of the poliovirus 1 by each concentration of FC was plotted. The constructs show that FC consistently inactivated poliovirus 1 more than 10 times as rapidly in drinking water as in purified water (Fig. 3).

DISCUSSION

The virucidal activity of FC is potentiated by KCl, NaCl, and CsCl at moderately alkaline pH levels where most of the FC exists as hypochlorite ion (OCl^-). In the presence of 0.05 to 0.1 M concentrations of these salts, FC is 10 to 30 times more virucidal than it is in the absence of salt (9, 12–16). At pH 9.0, the virucidal activity of FC is potentiated also in 0.007 M KCl (525 mg/liter) and probably in lower concentrations (2). Boric acid-NaOH buffer also potentiates the virucidal activity of FC at pH 9.0 (2), but to a lesser degree than KCl does. The virucidal activity of HOCl (pH 4.5) also appears to be potentiated by KCl, but perhaps to a lesser degree (2).

The occurrence of certain ions in a drinking water, if the ions are present in sufficient concentrations, probably potentiates the virucidal effectiveness of FC. Conversely, removal of those ions from a drinking water may significantly reduce the virucidal effectiveness of FC. It seems probable that many ions other than those in the salts thus far studied potentiate the virucidal activity of FC also. Drinking waters usually contain many different ions. The quantity of each in Cincinnati's drinking water (Table 1) may have been too small to have been responsible alone for the strong virucidal potentiation of FC. But it is possible that many of these ions individually potentiated the virucidal activity of FC and collectively produced the total potentiation that occurred.

The mechanism by which certain ions potentiate the virucidal effectiveness of FC is not yet clear. It has been suggested that ion pairs forming from salt cations and OCl^- may be more virucidal than OCl^- (10, 12, 17). It also seems

TABLE 1. Concentrations of ions and other components of Cincinnati drinking water (7)[a]

Test period and determination	Concn (mg/liter)									Total alkalinity (as $CaCO_3$) (mg/liter)	Total hardness (as $CaCO_3$) (mg/liter)	Total solids (mg/liter)	Turbidity[b]	pH
	Calcium (as Ca)	Magnesium (as Mg)	Chloride	Fluoride	Sulfate	Nitrate (as NO_3-N)	Iron (as total Fe)	Manganese (as total Mn)	Sodium					
1987														
Minimum monthly avg	38.0	5.3	18	0.95	37	0.79	<0.005	0.002	17.0	48	114	183	0.16	8.4
Maximum monthly avg	40.0	7.8	38	1.06	83	1.38	<0.005	0.002	17.0	66	154	299	0.22	8.8
Annual avg	39.0	6.6	26	1.00	59	1.13	<0.005	0.002	17.0	58	136	229	0.10	8.0
5 Yr														
Minimum monthly avg	32.0	5.0	16	0.20	34	0.79	<0.005	<0.001	9.3	45	113	176	0.03	8.0
Maximum monthly avg	51.0	12.7	45	1.06	129	1.64	0.019	0.007	34.5	72	187	308	0.23	8.9
Annual avg	40.8	8.4	25	0.94	74	1.19	0.009	0.003	17.5	56	138	229	0.14	8.5

[a] Revised and abstracted.
[b] Nephelometric turbidity units.

possible that salt cations, present in much greater numbers than the OCl^-, may neutralize negative charges on the virions that repel OCl^- and thereby facilitate OCl^- access to vital target sites within the virion. Other scenarios are also possible, of course.

In recent years, water treatment plant operators have modified treatment procedures to reduce the amounts of halomethanes and other toxic compounds (e.g., carcinogens and teratogens) produced in their drinking waters by the chlorination process. Although the U.S. Environmental Protection Agency has set no standards for the chlorination of drinking water supplies, some states do set minimum requirements or provide recommendations for FC-time couplings (11). Moreover, the quantities of chlorine applied to drinking waters are guided in part by laboratory studies of the rates at which FC inactivates viruses and other pathogens in water.

Although disinfection has been considered the main treatment barrier against all pathogens in drinking waters, we now know disinfection to be a flawed barrier. To wit, the cysts of giardia are highly resistant to FC and are generally removed by filtration, and the cysts of cryptosporidia appear to be too resistant to be inactivated by chlorination at all. The most FC-resistant pathogens that reasonably can be inactivated by chlorination are the viruses. Thus, if viruses are inactivated more rapidly in drinking waters (as they appear to be) than in the purified waters usually used in laboratory testing, it may make sense to reduce the quantities of FC applied to many water supplies. Such reductions in applied FC may reduce the amounts of halomethanes and other toxic substances produced in waters and, concomitantly, the long-term risk of cancer, teratogenesis, and other toxic consequences of chlorination.

Since it is important to minimize the quantities of FC applied to drinking waters to reduce the production of carcinogenic, teratogenic, and other toxic compounds that result from the chlorination process, it is important to determine which ions (or other substances in drinking waters) potentiate the virucidal effectiveness of FC and to what extent they do this.

It seems probable, especially since most drinking waters are alkaline, that it will be possible to reduce the quantities of FC applied to many drinking waters for disinfection purposes. It will probably be necessary, however, to determine at least the ionic content of a drinking water before an appropriate FC dosage can be determined. It is even possible that the virucidal potentiation of FC by each drinking water will need to be tested occasionally to determine the level of FC appropriate for it.

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