Viricidal Efficiency of Disinfectants in Water

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RECALLING the recent outbreak of waterborne infectious hepatitis in New Delhi, India (*1*), and the discovery in the past decade of more than 70 new human enteric viruses, it seems appropriate to review the information available regarding the viricidal efficiency of disinfectants in water. The term "enteric viruses" as used here includes infectious hepatitis virus, Coxsackie groups A and B, poliovirus, adenovirus, and the ECHO viruses. All of these are excreted in feces and can be found in urban sewage, especially in the late summer and early fall. Proved waterborne epidemics have occurred only with the virus of infectious hepatitis; however, outbreaks of poliomyelitis suspected of being waterborne have been reported (*2,3*). No waterborne diseases caused by Coxsackie and ECHO viruses have been recorded. No adenovirus infections have been attributed to drinking water, but three outbreaks of one of the clinical diseases caused by adenoviruses have been associated with swimming pools (*4–6*).

Chlorine

Because chlorine reacts with organic matter and forms combined chlorine compounds, reliable data on virus destruction depend on the chemical definition of the test water and determination of the types of titrable chlorine present, which are markedly influenced by the pH of the substrate. The rate of kill under fixed test conditions is proportional to the concentration of the kind of chlorine available. Sufficient demand-free virus material should be present to allow for accurate determination of the inactivation rate. Since many of the earlier studies on the destruction of viruses by chlorine did not meet these criteria, the resulting data are not included in this review. The reports before 1946 did not differentiate between free and combined chlorine, and many of the reports also showed such a high chlorine demand of the test system that the data on the viricidal efficiency of chlorine cannot be accurately interpreted.

Free Chlorine

Information on the viricidal efficiency of free chlorine in water is presented in the table. The amounts of free chlorine recorded in this table for the studies of Theiler's virus (*7*), infectious hepatitis virus (*8*), and type 2 poliovirus (*9, 10*) refer to the initial concentration. In each test the residual chlorine values were reduced to less than 1 mg./l. In the other investigations, the residual chlorine was not substantially less than the initial content (*11–14*).

The data of Weidenkopf (*12*) on poliovirus type 1 are probably most accurate because the test conditions are well defined, and the plaque count technique provides an accurate estimation of virus concentration. Taking into consider-

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ation differences in procedures of the several investigators, the information in the table reveals several significant points.

The data indicate that Theiler's virus is more resistant to the action of free chlorine than Coxsackie A2 virus, which is more resistant than poliovirus type 1. Adenovirus type 3 shows about the same resistance as does *Escherichia coli* (*14*). The comparative resistance of infectious hepatitis virus is not clearly shown because not enough contact times were tested. It is apparent, however, that the different viruses vary widely in their susceptibility to free chlorine. The pH exerts marked effects on the viricidal efficiency of free chlorine. Weidenkopf's tests (*12*) show that decreasing the pH from 7.0 to 6.0 reduces the required inactivation time by about 50 percent, and the findings of both Weidenkopf (*12*) and Clarke and others (*14*) indicate that a rise in pH from 7.0 to 8.8–9.0 increases the inactivation period about six times.

Viricidal efficiency of free chlorine in water

Virus [1]	Temperature (degrees centigrade)	Final pH	Free chlorine (mg./l.)	Virus destruction (percent/minutes)
Partially purified Theiler's virus in tapwater (Chang, et al., *7*).	25–27	6. 5–7. 0	4. 0–6. 0	98.6/10
	25–27	6. 5–7. 0	4. 0–6. 0	99/5
Feces-borne infectious hepatitis virus in distilled water (Neefe, et al., *8*).	Room	6. 7–6. 8	3. 25	([2])
Purified poliovirus 2 in distilled and lake water (Lensen, et al., *9, 10*).	19–25	7. 4–7. 9	1. 0–1. 5	([3])
Purified Coxsackie A2 in demand-free water (Clarke and Kabler, *11*).	3–6	6. 9–7. 1	. 58–. 62	99.6/10
	3–6	6. 8–7. 1	1. 9–2. 2	99.6/4
	3–6	6. 9–7. 1	3. 8–4. 2	99.6/2½
	3–6	8. 8–9. 0	1. 9–2. 0	99.6/24
	3–6	8. 8–9. 0	3. 7–4. 3	99.6/9
	3–6	8. 8–9. 0	7. 4–8. 3	99.6/5
	27–29	6. 9–7. 1	. 16–. 18	99.6/4
	27–29	6. 9–7. 1	. 44–. 58	99.6/3
	27–29	8. 8–9. 0	. 10–. 18	99.6/10
	27–29	8. 8–9. 0	. 27–. 32	99.6/7
	27–29	8. 8–9. 0	. 92–1. 0	99.6/3
Purified poliovirus 1 (Mahoney) in demand-free water (Weidenkopf, *12*).	0	6. 0	. 39	99.6/3½
	0	6. 0	. 80	99.6/1½
	0	7. 0	. 23	99.6/8
	0	7. 0	. 53	99.6/4½
	0	8. 5	. 53	99.6/16
	0	8. 5	1. 95	99.6/7½
	0	8. 5	5. 00	99.6/3
Purified poliovirus 1 (Mahoney) in demand-free water (Kelly and Sanderson, *13*).	25–28	7. 0	. 21–. 30	99.9/3
	25–28	9. 0	. 21–. 30	99.9/8
Purified poliovirus 3 (Saukett) in demand-free water (Kelly and Sanderson, *13*).	25–28	7. 0	. 11–. 20	99.9/2
	25–28	9. 0	. 11–. 20	99.9/16
Purified Coxsackie B5 in demand-free water (Kelly and Sanderson, *13*).	25–28	7. 0	. 21–. 30	99.9/1
	25–28	9. 0	. 21–. 30	99.9/8
	1–5	7. 0	. 21–. 30	99.9/16
	1–5	8. 0	. 21–. 30	99.9/30
Purified adenovirus 3 in demand-free water (Clarke, et al., *14*).	25	8. 8–9. 0	. 20	99.8/40–50 sec.
	25	6. 9–7. 1	. 20	99.8/8–16 sec.
	4	8. 8–9. 0	. 20	99.8/80–100 sec.
	4	6. 9–7. 1	. 20	99.8/8–10 sec.

[1] Figures in parentheses are reference numbers.
[2] 30 minutes contact time protected all of 12 volunteers.
[3] 10 minutes contact time protected all of 164 inoculated mice.

In the destruction of viruses by chlorine, Clarke's work (*14*) suggests that the temperature coefficient for a 10° change (Q_{10}) is in the range of 2 to 3, indicating that the inactivation time must be increased 2 to 3 times when the temperature is lowered 10°C. Data in the table also indicate that the chlorine concentration coefficient (N) lies in the range of 0.7 to 0.9. This means that the inactivation time is reduced a little less than half when the free chlorine concentration is doubled. To increase virus kill, therefore, there is some advantage in increasing the contact time instead of raising the chlorine content.

Kelly and Sanderson (*13*) also observed that the viricidal efficiency of free chlorine is dependent on pH, temperature, chlorine concentration, and exposure time. In addition, they pointed out that complete inactivation of some enteric viruses cannot be attained by exposure to 0.2 mg./l. of chlorine for 10 min. at pH 7.0 (usual bacterial parameters of disinfection) at an initial level of 300 to 3,000 virus doses per milliliter in the water.

With low chlorine residuals, the virus inactivation rate is markedly affected by temperature and pH. Studies indicate that at a temperature of approximately 20° C. and pH values no higher than 8.0–8.5, a free chlorine residual of 0.2 to 0.3 mg./l. will probably destroy most of the tested viruses in 30 minutes. At temperatures below 10–15° C. and pH values greater than 8.5, effective virus kills with free chlorine residuals of 0.2 to 0.3 mg./l. are probably not attainable without long detention periods.

Combined Chlorine

Trask and others (*15*) found that 5.4 mg./l. of residual chlorine as chlorine-ammonia was required to destroy 90 percent of Theiler's virus (FA strain) in 30 minutes, and 8.1 mg./l. was required for the same destruction in 10 minutes, when the weight ratio of chlorine to ammonia was 1 to 4. The temperature and pH of the treated water were not stated. At 26° C., a pH of 6.8 to 7.1, and a chlorine-ammonia weight ratio of 2 to 3, Chang and co-workers (*7*) observed that 5.2 mg./l. of residual chlorine killed only 85.7 percent of Theiler's virus in 60 minutes. Trask and associates (*15*) also stated that 12.1 mg./l. of titrable chlorine as azochloramid destroyed 90 percent of Theiler's virus in 30 minutes, and Fair and associates (*16*) found that 18 to 20 mg./l. of titrable chlorine as monochloramine T killed 60 to 80 percent of Theiler's virus in 60 minutes at 27° C. and pH of 7.0 to 7.2. These data clearly show the low viricidal efficiency of these compounds.

Kelly and Sanderson (*17*) showed that poliovirus (type 1, MF 500 and Mahoney strains) and Coxsackie virus (group B, type 5, EA 80) in water were inactivated by combined residual chlorine, the effective concentration depending on pH, contact time, and strain of virus. In general, longer contact was required to destroy type 1 poliovirus than Coxsackie B virus in this study, and increasing the pH decreased the rate of inactivation. At 25° C. and a pH of 7, a concentration of at least 9 mg./l. was necessary for inactivation of poliovirus with a contact period of 30 minutes, and of 6 mg./l. with a 1-hour contact period. A concentration of 0.5 mg./l. required a contact period of more than 7 hours. These data indicate that present methods of disinfecting water with combined chlorine may not be adequate to destroy the more resistant virus strains.

Chlorine Dioxide

Ridenour and Ingols (*18*) found chlorine dioxide to be as effective as free chlorine in inactivating poliovirus if the residuals are measured by the orthotolidine-arsenite (OTA) method. In 1953, Hettche and Schulz-Ehlbeck (*19*) reported that 0.08 mg./l. chlorine dioxide, measured by the orthotolidine (OT) method, showed the same viricidal activity for poliovirus as 0.15 mg./l. ozone or 0.25 mg./l. free chlorine. However, Post and Moore (*20*) report that the OT and OTA methods make no distinction between chlorine and chlorine dioxide. Critical comparison of data on the viricidal efficiency of free chlorine and chlorine dioxide is therefore impossible if OT or OTA methods are used to measure the chlorine dioxide content.

Iodine

Recent reports (*21, 22*) indicate that different enteric viruses may exhibit varying resistances to iodine and that Coxsackie A2 and B1 viruses have about the same resistance to molecular iodine, but that these two viruses have twice the resistance of poliovirus type 1 or ECHO 7 virus.

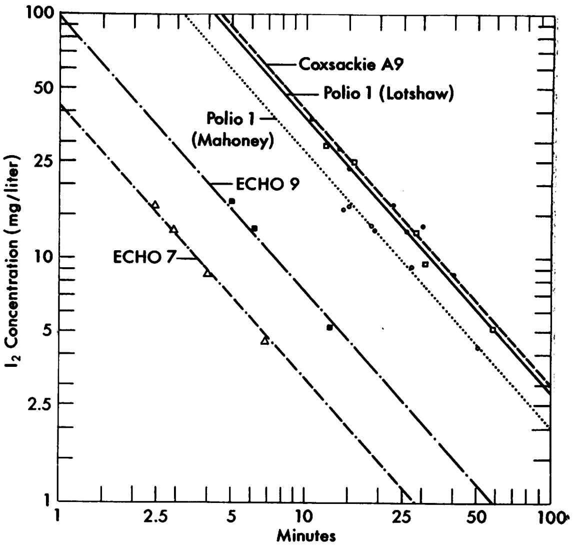
Berg and associates (*23*) studied the destruction of several enteric viruses by elemental iodine (I_2) under carefully controlled conditions. The chart shows the relationship of contact time and I_2 concentration required for 99 percent destruction of each of five viruses tested at 15° C. The slopes of all the lines are about the same and indicate that a change in contact time will have a slightly greater effect than a proportional change in the iodine concentration. Coxsackie A9 virus was the most resistant virus tested, requiring 10 mg./l. of I_2 to destroy 99 percent of the agent in 34 minutes at 15° C. At the same temperature and I_2 concentration, 99 percent of ECHO 7 virus was killed in 3½ minutes. Poliovirus type 1, Lotshaw strain, was almost as resistant as Coxsackie A9 virus, while the resistance of poliovirus type 1, Mahoney strain, and ECHO 9 virus fell between the two extremes. With essential conditions constant, about 10 times longer contact time was required to destroy the most resistant virus as was needed to destroy the least resistant virus tested. In other comparative tests, at 25° C., 99 percent of Coxsackie A9 virus was destroyed in 52 minutes by 1.0 mg./l. of I_2, while the same concentration of I_2 (extrapolated data) destroyed 99 percent of *E. coli* in only 0.03 minute. Thus the contact time for the Coxsackie virus was more than 1,700 times that for the *E. coli*.

Iodide and iodate ions are completely inert as viricides. Tri-iodide is either inert or acts so slowly that it is ineffective under most test conditions. Hypoiodous acid, which increases in concentration as the pH of the water increases, appears to be a more potent viricide than I_2.

Ozone

Kessel and co-workers (*24*) reported that 0.05 to 0.45 mg./l. of ozone destroyed the same quantity of poliovirus in 2 minutes as did 0.5 to 1.0 mg./l. of residual gaseous chlorine in 1½ to 3 hours. Because a crude-virus suspen-

Relationship of contact time and concentration of iodine (I_2) at 15° C. required for 99 percent destruction of five viruses



sion was used in the test and the types of residuals were not differentiated, it seems likely that most of the residual chlorine was in the combined form. The findings of Hettche and Schulz-Ehlbeck (*19*) also indicated that, on a weight basis, ozone was somewhat more viricidal for poliovirus than was free chlorine.

Ultraviolet Light

The studies of Habel and Sockrider (*25*), Murray and co-workers (*26*), Milzer and co-workers (*27*), and Oppenheimer and co-workers (*28*), have shown that many viruses and vaccines including enteroviruses in liquid suspensions can be inactivated by ultraviolet irradiation. Carlson and associates (*29*) found that ultraviolet irradiation was more effective in destroying poliovirus in water than coagulation and sedimentation, filtration, activated charcoal, or storage, although their results cannot be considered a quantitative measure of the effectiveness of these methods. Coxsackie A virus and Theiler's virus in water were more resistant than *E. coli* to ultraviolet light in studies by Gilcreas and Kelly (*30*). One major difficulty in ultraviolet light sterilization of water is the lack of a rapid field test to determine whether the water has been adequately treated. It is therefore necessary that the treatment unit be equipped with an internally situated ultraviolet energy source that supplies a stable energy application and that this incident energy penetrate the fluid and provide an essentially uniform density throughout the fluid as it passes through the effective radiation area.

Summary

Data from recent studies of the efficiency of various disinfectants in inactivating enteric viruses in water appear to support the following summarizations:

1. Different types of enteric viruses vary widely in the degree of resistance to free chlorine. Poliovirus, Coxsackie, and some ECHO viruses seem to be more resistant than coliform or enteric pathogenic bacteria. The free chlorine residuals required for inactivation depend on pH, temperature, and contact time.

2. Combined chlorine is considerably less viricidal than free chlorine, requiring higher concentrations or longer contact periods to achieve comparable inactivation.

3. Iodine is an effective viricide, but requires greater residuals and longer contact than hypochlorous acid.

4. Chlorine dioxide, ozone, and ultraviolet light may be useful disinfectants; however, their efficiency in water in comparison with that of free chlorine and the quantitative effects of pH and temperature have not been established.

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