Elemental Iodine as a Disinfectant for Drinking Water

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The germicidal efficiency of dilute aqueous iodine has been evaluated to determine the usefulness of elemental iodine as an emergency disinfectant for drinking water. Iodine concentrations of 5 to 10 p.p.m. have been found effective against all types of water-borne pathogenic organisms, including the enteric bacteria, amebic cysts, cercariae, leptospira, and viruses, within 10 minutes at room temperature. High germicidal activity is maintained over the pH range 3 to 8 in the presence of a variety of water contaminants. Waters with high organic color (>50 p.p.m.) may exhibit sufficient iodine demand to require an increased dosage for satisfactory disinfection. At low temperatures the germicidal action is slowed, so that a contact time of 20 minutes is required in near-freezing waters. Iodine has a number of advantages as an emergency disinfectant for water supplies as compared with hypochlorites or chloramine-type materials. Its germicidal action is less dependent on pH, temperature, and time of contact, nitrogenous impurities do not impair its effectiveness, and side reactions leading to consumption of the germicide are less marked for iodine than for chlorinous disinfectants. Almost all waters can be made safe for drinking with a single dose of iodine of approximately 8 p.p.m.

ALTHOUGH iodine is well known as a germicide for medical application and has often been recommended for the treatment of water supplies under emergency conditions, little information has been published on its efficacy in water treatment or the concentrations required (*6*). An extensive program of research on the germicidal properties of dilute aqueous solutions of iodine has now shown that iodine is an effective germicidal agent at concentrations of a few parts per million and that it can be advantageously employed for the emergency treatment of natural waters of varying quality.

To be useful in emergency disinfection of water a substance must be effective not only against the water-borne pathogenic bacteria—the typhoid, paratyphoid, cholera, and bacillary dysentery organisms—but also against other water-borne pathogens, including *Entamoeba histolytica, Leptospira icterohaemorrhagiae,* cercariae of *Schistosoma* sp., and viruses. All these types of infectious agents have been considered in these studies. Investigations of the effects of temperature, pH, color, turbidity, nitrogenous material, and other possible variations in water quality on the germicidal effectiveness of iodine have also been included in order to establish its general usefulness as a water disinfectant.

Studies on Bacteria

Most of the experiments on the bactericidal qualities of aqueous iodine solutions were conducted with the common test organism, *Escherichia coli*. However, a sufficient number of experiments were also carried out with the enteric pathogens themselves to establish the validity of the results obtained with *E. coli*. The general method of testing was to add the desired numbers of organisms to continuously stirred gallon quantities of appropriate water or solution and, following the addition of suitable concentrations of iodine solution, to withdraw samples at specified times, adding them immediately to sufficient sodium thiosulfate solution to reduce the iodine present. Surviving organisms were then determined either by plating or by suitable fermentation tests in tubes after appropriate dilution of the reduced samples.

Attempts to evaluate the fundamental bactericidal behavior of iodine in pure water were not too successful. A high degree of variability was encountered, probably because the concentrations of iodine required to give measurable rates of kill were so small that they could be completely neutralized by mere traces of reactive impurities. In some tests, concentrations of iodine as low as 0.02 p.p.m. exhibited rapid bactericidal action, while in other test at the same concentration there was no measurable killing. However, when iodine concentrations of 0.05 p.p.m. or more were used, 10^4 *E. coli* per ml. were consistently reduced to less than one per milliliter within 10 minutes at room temperature. The fundamental bactericidal activity of iodine in water is thus about as great as that of hypochlorous acid.

Results of experiments under conditions more nearly approximating those likely to be encountered in the practical use of iodine are shown in Table I. The initial concentrations of bacteria, 10^6 per ml., are considerably higher than those expected even in grossly polluted waters, and so the iodine concentrations found to be bactericidal in these tests should be more than adequate in natural waters. It was considered that satisfactory disinfection was achieved under the stringent conditions of these tests and those subsequently presented when the numbers of viable bacteria had been reduced below 5 per 100 ml. At 25° C. in acid solution a free chlorine residual of about 2 p.p.m. is required to meet this criterion.

The data in Table I indicate that iodine concentrations of the order of 2 to 5 p.p.m. are adequate for the destruction of bacteria in water at 25° C. with a 10-minute contact period.

Table I. Bactericidal Efficiency of Iodine in Cambridge Tap Water

Temp., 25° C. pH, 8.1 to 8.5. Test organism, *E. coli*. Initial concentration, N_0, 10^6 per ml.

I_2 Concn., P.P.M.		Viable Organisms per 100 Ml., MPN, after:			
Initial	30 min.	5 min.	10 min.	20 min.	30 min.
1.0	0	1600	540	240	130
2.0	1.2	4.6	1	1	2.2
3.0	1.6	4.6	4.6	4.6	< 1
4.0	2.6	24	6.9	2.5	1
5.0	3.3	< 1	< 1	< 1	< 1

Further tests were conducted using a standard iodine dosage of 7 to 8 p.p.m. This concentration was selected so that bactericidal residuals of 4 to 5 p.p.m. might be obtained in the presence of iodine demands somewhat greater than those encountered in Cambridge tap water. In addition, the use of such a dosage is indicated to be necessary for the destruction of cysts of *E. histolytica*.

The effectiveness of this dosage of iodine against various species of bacteria is presented in Table II. All of the pathogenic enteric bacteria, with the exception of *V. comma*, exhibit about the same sensitivity to iodine as does *E. coli*. The cholera organisms are definitely more easily killed. Moreover, as is shown by the last line of Table II, iodine is about as effective against the natural *coli-aerogenes* organisms of sewage as it is against the culture-produced organisms.

Table II. Destruction of Various Types of Bacteria by Iodine

Cambridge tap water (10% sea water added for *V. cholera* tests; 10% filtered Cambridge sewage added for *coli-aerogenes* tests). Iodine dosage, 7–8 p.p.m.; residuals after 10 min., 5–7 p.p.m.; residuals after 30 min., 4–6.5 p.p.m. Temp, 25° C. pH values range from 4.5 to 8.1; low values obtained by addition of citric acid, high values by addition of $NaHCO_3$. Initial No. of organisms, 10^6 per ml.

Organism	Geometric Mean No. of Survivors per 100 Ml. after: 5 min.	10 min.	20 min.	30 min.
E. coli (22 tests)	6.6	2.4	1.6	1.3
Sal. typhosa (8 tests)	1.4	< 1	< 1	< 1
Sh. dysenteriae (9 tests)	1.3	1	1	< 1
Sal. schöttmuelleri (9 tests)	21	5.1	2.7	2.1
Vibrio cholera (6 tests)	< 1	< 1	< 1	< 1
Mixed *coli aerogenes* flora of sewage, initial count 5000 per ml. (3 tests)	1.7	1.3	1.3	1.6

Individual experiments included in the means of Table II were conducted at pH values ranging from 4.5 to 8.1; over this range no effect of pH on the bactericidal efficiency of iodine was observed. Similar killing action was also obtained in experiments, not included in Table II, in which pH values were 9.5 to 10. This behavior is in marked contrast to the germicidal action of chlorine, which is well known to be strongly dependent on pH.

The effects of a number of the impurities encountered in natural waters on the bactericidal action of iodine are shown in Table III.

Table III. Effect of Additives on Germicidal Efficiency of Iodine in Water

Cambridge tap water used as basic medium. Iodine dosage, 6–8 p.p.m. Temp., 25° C. Test organism, *E. coli*. Initial concentration, 10^6 per ml.

Additive	pH Range	Geom. Mean No. of Survivors per 100 Ml. after: 5 min.	10 min.	20 min.	30 min.	Approx. Range of Residuals
1–5 p.p.m. NH_3 N (8 tests)	6.4–9.8	4.3	1.7	1	1.2	6.5–5.0
1–5 p.p.m. urea N (10 tests)	6.3–6.6	3.6	1.3	1	1.2	6.5–5.5
50–500 p.p.m. of various clays (14 tests)	6.3–6.5	6.9	2.6	1.1	1.6	7.0–5.7
35 p.p.m. loess	6.1	2.5	1.3	1	22	7.0–6.4
57 p.p.m. loess	6.1	6.9	4.2	< 1	4.6	7.0–6.4
165 p.p.m. loess	6.1	110	92	4.6	7.4	6.5–6.4
245 p.p.m. loess	6.1	350	240	11	1	7.0–6.4

Ammonia nitrogen and urea nitrogen in concentrations up to 5 p.p.m. had no measurable effect on the efficiency of disinfection. Similarly, turbidity produced by most of the clays tested was without effect, but the presence of high concentrations of fine loess appeared to cause a decrease in bactericidal action. The tests with this material are, therefore, shown separately in Table III. No explanation for the action of this fine sand is apparent.

The presence of organic color in water is known to be one of the major causes of chlorine demand in water, with consequent increase in the dosage requirements for bactericidal action. It was of interest to determine whether the action of iodine was affected similarly. Therefore, a series of highly colored waters was collected and tests were conducted using these waters as the reaction medium. Later it was found that the behavior of these colored waters could be simulated by dilute tea and this material was also employed in a number of tests. Table IV, which presents results of these tests, shows that organic color does produce iodine demand and that in strongly colored waters a dosage of 7 to 8 p.p.m. may provide insufficient treatment because of rapid reduction of the iodine. However, doubling the dose so as to give residuals comparable with those found previously in the tests with uncolored water leads to satisfactory bactericidal action, as shown in the second section of Table IV.

Table IV. Effect of Organic Color on Bactericidal Efficiency of Iodine in Water

Temp., 25° C. Test organism, *E. coli*. Initial concentration, 10^6 per m..

Description of Water	Color	pH	No. of Survivors per 100 Ml. after: 5 min.	10 min.	20 min.	30 min.	Residual
			Iodine Dosage 7 to 8 P.P.M.				
Lily pond	60	6.6	35	70	14	22	3.5–2.9
Hobbs Brook (4 tests)	90	5.3	14	5	3.4	1.3	3.5–2
Charles River	80	6.4	6.8	< 1	6.8	2	3.7–1.5
Tea infusion	~70	5.9–6.5	280	78	69	62	1.8–1.3
			Iodine Dosage 14 to 16 P.P.M.				
Hobbs Brook (4 tests)	90	5.0	2.9	2.7	1.7	1	9.0–7.5
Charles River	80	6.2	3.7	2.0	< 1	2.0	8.3–6.6
Tea infusion (5 tests)	~70	6.4	3.8	3.6	2.0	1.6	8.0–6.0

Because disinfection is a rate process, it is to be expected that the bactericidal activity of iodine will be exerted more slowly at lower temperatures. This is illustrated by Table V, in which results at low temperatures are presented along with previous data for 25° C. Whereas at room temperature a contact time of 20 minutes is sufficient to meet the adopted standard for satisfactory bactericidal action, at low temperatures a 20-minute period is required.

Table V. Effect of Temperature on Bactericidal Action of Iodine in Water

Test organism, *E. coli*. Initial concentration, 10^6 per ml. Iodine dosage 7 to 8 p.p.m.

Temp., ° C.	pH	Geom. Mean No. of Survivors per 100 Ml. after: 5 min.	10 min.	20 min.	30 min.	Av. Residual
2–3 (5 tests)	6.2–6.7	50	22	3.2	5.4	7.1–6.2
7 (6 tests)	5.7–7.4	14	5.4	3.5	1	7.5–6.8
25 (22 tests)	4.5–8.1	6.6	2.4	1.6	1.3	6.7–5.7
Test with 10% Sewage in Cambridge Tap Water, Initial *coli aerogenes* Approximately 3000 per Ml.						
7–9° (3 tests)	6.0–6.6	2.6	2.2	1.4	1	6.3–5.5

Studies on *Entamoeba Histolytica*

The causative agent of amebic dysentery, *E. histolytica*, exists in two forms: as active trophozoites or in the resting stage as cysts. The trophozoites are very sensitive to the action of iodine, less than 0.1 p.p.m. being sufficient to cause destruction within 5 minutes at ordinary temperatures. On the other hand, the cysts, which are the water-borne carriers of the disease, are very resistant to the action of germicides, resembling bacterial spores in this respect. Because carrier rates are high in most parts of the world, the cysts are of wide occurrence in polluted waters and a suitable germicide for emergency use must have cysticidal as well as bactericidal properties.

The techniques employed for the study of cysticidal activity have been reported by Chang (*1*).

The desired numbers of cysts were added from a concentrated suspension to a large volume of appropriately conditioned water and then 500 ml. was dispensed into each of a series of test bottles. Each bottle was dosed with a suitable amount of iodine solution, the amounts for different bottles being graded to include the expected minimum cysticidal concentration, and was shaken in a water bath at a fixed temperature for the desired contact period. Then, after removal of a portion of the solution for chemical testing, sufficient sodium sulfite was added to reduce the iodine present, and the entire sample was centrifuged to concentrate the cysts. The sediment was subjected to appropriate culture techniques to determine the viability of the collected cysts.

A test was considered complete and valid, only when the bottles with lower iodine concentrations gave positive cultures, while those with higher concentrations showed no surviving cysts. By this procedure minimum cysticidal levels were established within about 10%.

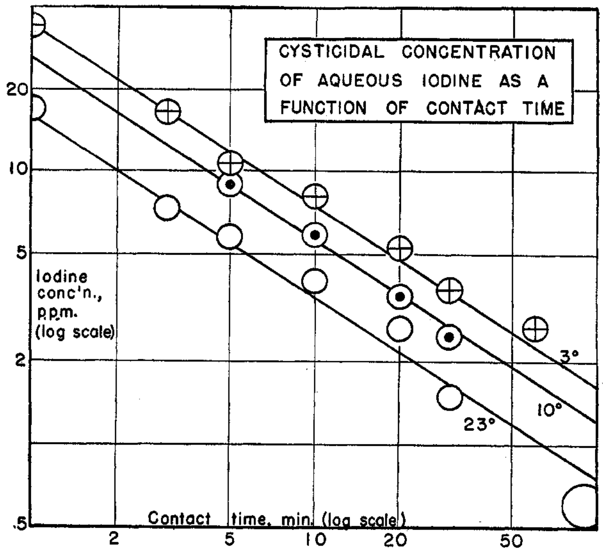

Figure 1. Cysticidal Concentration of Aqueous Iodine as a Function of Contact Time

Figure 1 shows the minimum cysticidal concentrations of elemental iodine as a function of time of contact for temperatures of 3°, 10°, and 23° C. at pH 5 for an initial cyst concentration equal to 30 per ml. The results conform to an equation of the type, $C^n t = k$, where $n = 1.5$ and $k = 200$, 130, and 65 for 3°, 10°, and 23° C., respectively. A value of $n = 1.0$ was previously obtained for the destruction of amebic cysts by hypochlorous acid (*3*). Consequently, concentration level is relatively more important and time of contact is relatively less important in the destruction of cysts by iodine as compared with hypochlorous acid. This is just the reverse of the behavior of *B. metiens* spores with these two germicides, even though the required concentrations of killing agent are about the same for both types of organisms. Thus, Weber and Levine (*7*) found $n = 1.0$ for the action of hypochlorous acid and Marks, Wyss, and Strandskov (*5*) found $n = 0.74$ for the action of iodine on spores of *B. metiens*. As no general theory has been advanced to account for the various values of n encountered in germicidal work, no explanation of this peculiar difference can be given. However, the results point up the difficulties encountered in attempting to generalize the germicidal behavior of a substance on the basis of experiments with one type of organism.

The effects of temperature and of pH on the cysticidal action of iodine for a contact period of 30 minutes are shown in Figure 2. The scattering of points for pH values 3, 5, and 7 indicates that the cysticidal activity of iodine is independent of pH over this range. From the slope of the straight line drawn through the points, an activation energy $E = 9500$ cal. is obtained by means of the formula

$$\frac{-\,d \log C}{d\,\frac{1}{T}} = \frac{E}{2.3\,nR}$$

The factor, n, enters this equation because the data refer to concentrations required to kill in a given time; the customary formula for E requires that the data be times required for kill at a specified concentration. The corresponding Q_{10} value at 25° is 1.65.

Again there is a difference between the comparative action of iodine and hypochlorous acid on amebic cysts and on bacterial spores. The cysticidal action of iodine is less strongly dependent on temperature than is that of hypochlorous acid ($E = 13{,}000$ cal.), whereas Wyss and Strandskov (*8*) found the sporicidal action of iodine ($E = 22{,}000$ cal.) to be much more dependent on temperature than that found for hypochlorous acid ($E = 12{,}000$ cal.) by Weber and Levine (*7*).

It is desirable that an emergency disinfectant accomplish its action within 10 minutes. Examination of Figure 1 shows that the necessary concentration of iodine for effective action against cysts within this period is approximately 5 p.p.m. in clean waters that are not too cold. The applied dose of iodine should be somewhat higher to allow for the iodine demands of most natural waters and for other possible impurities. It was concluded that a suitable dose of iodine for emergency disinfection is about 8 p.p.m.

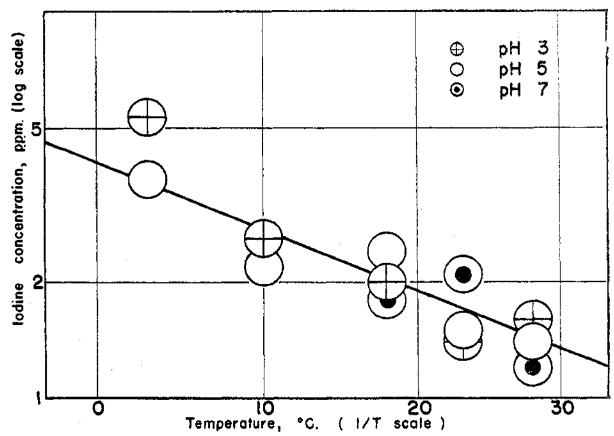

Figure 2. Effect of pH and Temperature on Cysticidal Concentration of Iodine

Contact time 30 minutes

Practical tests with various natural and synthetic waters, using a standard dosage of 8 p.p.m. of iodine, led to the following conclusions:

The standard dosage of 8 p.p.m. exerts cysticidal action within 10 minutes at 23° C. in waters containing up to 400 p.p.m. of alkalinity, 1000 p.p.m. of salt, 5 p.p.m. of urea or ammonia nitrogen, 10% of Cambridge sewage, 600 p.p.m. of soil turbidity, or moderate amounts of organic color. The standard dosage is also effective up to pH 7.7 or greater.

In waters with high organic color ($> \sim 70$ p.p.m.) or with demands greater than about 3 p.p.m. an increased dosage of iodine is necessary. Twice the standard dosage was found to be effective in all waters tested.

At low temperatures (0° to 5° C.) a contact time of 10 minutes is insufficient. At these low temperatures, 20 minutes was required for complete cysticidal action.

Other Types of Infectious Agents

The only virus disease that has been shown definitely to be transmitted by means of water is infectious hepatitis. Experimental work on the virus of this disease was impractical because the only known test subject is man. Therefore, as the virus of poliomyelitis is known to survive in water and may possibly be transmitted by this route, it was selected as the test organism for studies on the viricidal action of aqueous iodine.

The Lansing, mouse-adapted strain of human poliomyelitis virus was used. The brains of infected mice were well emulsified and centrifuged, and after the potency of the supernatant fluid had been established by intracerebral inoculations into suckling mice, appropriate dilutions were made in sterile tap water buffered at pH 9. Experiments were conducted by mixing portions of this solution with equal quantities of sterile tap water containing an appropriate amount of iodine. After suitable contact periods samples were removed, the iodine present was reduced with thiosulfate, and then 0.03-ml. portions were injected into 10 laboratory mice. Two simultaneous control groups of mice received injections of untreated test solutions, one of which had been dosed with thiosulfate.

Table VI. Inactivation of Poliomyelitis Virus by Aqueous Iodine

Virus Dilution	Initial Dosage of Iodine, P.P.M.	Contact Time, Min.	Death Rate of Mice, % Treated	Death Rate of Mice, % Untreated[a]	Residual Iodine, P.P.M.
1:500	7.5	5	10	50	. . .
		10	20	90	. . .
		20	0	70	3.3
1:100	7.5	5	70	100 (100)	. . .
		10	70	60	0
		20	80	40	0
1:500	25	5	33	70 (100)	4.5
1:500	45	5	0	70 (100)	17.5
1:100	25	5	10	80 (90)	0.5
1:100	25	5	30	70 (70)	1.5
1:100	50	5	20	70 (70)	16.0

[a] Values in parentheses are controls without thiosulfate.

The results obtained, shown in Table VI, are neither very complete nor very conclusive. This is partly because of the difficulties inherent in all virus work, and partly because of inability to free the test solutions from colloidal material protecting virus particles or from materials exhibiting strong iodine demand. However, the results indicate that iodine does have viricidal properties and suggest that it is effective at the concentration necessary for the destruction of amebic cysts.

Another known water-borne infectious organism is *Leptospira icterohaemorrhagiae*, the causative agent of Weil's disease or infectious jaundice. Studies on this organism, which have been reported in detail (*2*), showed that leptospira were inactivated within 5 minutes at room temperature by a 10 p.p.m. iodine dosage which gave a 0.5 p.p.m. residual. The large iodine demand, due to organic contamination that could not be separated from the leptospira cultures, makes it difficult to set a specific minimum iodine residual for destruction of leptospira. However, because almost all the demand was exerted within the first 30 seconds, it seems probable that the 0.5 p.p.m. residual iodine more nearly represents the concentration required to destroy leptospira in water under natural conditions. The authors judge from their results that the resistance of leptospira to iodine is about the same as that of the enteric bacteria.

Studies of the effect of iodine on cercarieae of the schistosomes, the causative agents of schistosomiasis, have been conducted by Jones and Brady (*4*) at the National Institutes of Health. They found that at room temperature a dosage of 7.5 p.p.m. of iodine was effective in inactivating *Schistosoma* cercariae in 3 to 5 minutes in raw Potomac River water. When leaf extract was added to this water, dosages of 7.5 and 15 p.p.m. of iodine were only occasionally effective in killing the cercariae in a short time. However, because no data on iodine residuals or demands are available for these waters, the iodine may all have been reduced before any cercaricidal action could occur.

Discussion and Summary

These studies have shown that iodine is a suitable agent for the emergency disinfection of water supplies, for it is effective against all types of pathogenic organisms within a reasonable time at a concentration of a few parts per million. It has a number of advantages over aqueous chlorine or compounds that are germicidal by virtue of liberated hypochlorous acid. Most important is its ability to maintain substantially constant germicidal efficiency in waters with high pH values and in waters containing ammonia or other nitrogenous impurities. The facts that the action of iodine depends less on contact time or on temperature than does that of chlorine are minor advantages. Iodine and chlorinous disinfectants suffer alike in the presence of organic color or other reducing materials. The only solution to this problem for either type of germicide is the use of a sufficiently large dosage to leave an effective residual after the demand has been satisfied.

The concentration of aqueous iodine necessary for satisfactory emergency treatment is primarily determined by the concentration required to kill the cysts of *E. histolytica*. It has been found that a dosage of 8 p.p.m. of iodine will give complete destruction of 30 cysts per ml. within 10 minutes in most natural waters, exceptions being waters with temperatures near 0° C. or with iodine demands greater than 4 p.p.m. The iodine dosage also reduces 10^6 enteric bacteria per ml. to less than 5 per 100 ml. within 10 minutes and is effective against leptospira, schistosomes, and viruses. Adequate treatment of highly colored waters—those with demands greater than about 4 p.p.m.—can be obtained by increasing the iodine dosage; for waters near 0° C. satisfactory disinfection can be secured by increasing the time of treatment to 20 minutes.

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