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Virucidal effect of chlorinated water containing cyanuric acid

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SUMMARY

The inhibitory influence of cyanuric acid on the virucidal effect of chlorine was studied. The time required for 99·9% inactivation of ten enteroviruses and two adenoviruses by 0·5 mg/l free available chlorine at pH 7·0 and 25 °C was prolonged approximately 4·8–28·8 times by the addition of 30 mg/l cyanuric acid. Comparative inactivation of poliovirus 1 by free available chlorine with or without cyanuric acid revealed the following. The inactivation rate by 1·5 mg/l free available chlorine with 30 mg/l cyanuric acid or by 0·5 mg/l free available chlorine with 1 mg/l cyanuric acid was slower than by 0·5 mg/l free available chlorine alone. Temperature and pH did not affect the inhibitory influence of cyanuric acid on the disinfectant action of chlorine. In the swimming-pool and tap water, cyanuric acid delayed the virucidal effect of chlorine as much as in the 'clean' condition of chlorine-buffered distilled water. The available chlorine value should be increased to 1·5 mg/l when cyanuric acid is used in swimming-pool water.

INTRODUCTION

Cyanuric acid and cyanurates have been used in swimming-pool water to increase the stability of chlorine in the presence of sunlight. Cyanuric acid itself has been shown to be a material of low acute and chronic toxicity (Canelli, 1974). It is generally agreed that a cyanuric acid concentration 20–30 mg/l is required before stabilization of the chlorine residue is achieved (Nelson, 1967). The effect of cyanuric acid on the bactericidal action of chlorine has been studied by several investigators. In laboratory experiments with distilled water, cyanuric acid decreases the bactericidal activity of chlorine (Andersen, 1965; Fitzgerald & DerVartanian, 1967; Robinson & Mood, 1967). Studies using swimming-pool water and actual pool operation data, however, indicate that cyanuric acid does not delay the bactericidal effect of chlorine (Kowalski & Hilton, 1966; Morgan, Gilcreas & Gubbins, 1966; Swatec, Raj & Kalbus, 1967). Based on the accumulated findings of these reports, Canelli(1974) concludes that adequate disinfection may be obtained with cyanuric acid concentrations of 20–25 mg/l with free available chlorine concentrations in the range of 1·0–1·5 mg/l. It has been recommended in Japan that the swimming-pool cyanuric acid concentration is maintained below 50 mg/l, with free available chlorine concentration in the range 0·4–1·0 mg/l.

There is no report of the effect of cyanuric acid on the virucidial effect of chlorine. In the present report, enteroviruses and adenoviruses are used to show

that cyanuric acid also reduces the virucidal effect of chlorine in chlorine-demand-free buffered distilled water. A study is undertaken to confirm the effect of cyanuric acid on the inactivation of poliovirus type 1 by chlorine. Specifically, this study examines the effects of pH, water temperature and actual swimming-pool water on the virucidal effect of chlorine.

MATERIALS AND METHODS

Preparation and purification of stock viruses

The ten enteroviruses and two adenoviruses used on this study, poliovirus 1 (Sabin) and 2 (Sabin), coxsackievirus A24 (EH24/70), coxsackievirus B3 (Nancy), B4 (JVB) and B5 (Faulkner), echovirus 6 (D'Amori), 7 (Wallace) and 11 (Gregory), enterovirus 70 (AHC(J670/71)) and adenovirus 3 (G.B.) and 7 (Gomen), were obtained from the National Institute of Health, Tokyo. Coxsackievirus A24, echovirus 11 and enterovirus 70 were prepared in RD-18S cells cloned from RD cells (Sakae *et al.* 1985), and the other enteroviruses were prepared in HeLa cells maintained with Hanks' balanced salt solution (HBSS). After the appearance of cytopathic effect, the supernatant was centrifuged at 3000 g for 30 min and the supernatant was used as crude viral suspension for further purification. Adenoviruses were prepared in HEL cells maintained with Eagle's minimum essential medium plus 2% fetal calf serum. After the appearance of cytopathic effect, the cells containing progeny viruses were scraped from the plates, washed three times with HBSS, resuspended in HBSS equal to the original volume of the maintenance medium, sonicated, centrifuged at 3000 g for 30 min, and the supernatant was used as crude viral suspension for purification. Enterovirus titres were determined in the same cell line, and those of adenoviruses were determined in Chang conjunctiva cells by plaque technique. The titres of all virus preparations were 10^6–10^8 p.f.u./ml. The procedures for purification of all viruses to reduce the chlorine demand of the preparation were the same as those reported by Payment, Tremblay & Trudel (1985).

Preparation of suspending medium and glassware

Chlorine demand-free (CDF) buffer was used as a suspending medium in the inactivation experiments at pH 6·0, 7·0 and 8·0. A 0·05 M sodium phosphate buffer was prepared with distilled water containing sodium hypochlorite to obtain 5 mg/l free available chlorine. After storage at room temperature for 2 days, this chlorinated buffer was exposed to ultraviolet light overnight and then analysed for absence of chlorine by the orthotolidine test. Actual water samples from an indoor swimming pool and tap water in Seto city in Aichi Prefecture were superchlorinated with 5 mg/l free available chlorine for 2 days and exposed to ultraviolet light overnight before testing. A partial chemical analysis of these samples was made following the method recommended by the Japan Water Works Association.

All glassware was immersed in distilled water containing 10 mg/l free available chlorine for 2 days, rinsed several times in CDF water, and sterilized with hot air.

Experimental procedure

Experiments were carried out in duplicate in a constant-temperature water bath. One hundred and ninety-nine ml of a suspending medium containing the appropriate concentration of cyanuric acid was placed in 300 ml Erlenmeyer flask. Then 1 ml of the virus preparation to be tested was added and mixed at 100 rev./min with a magnetic stirrer. After mixing, 1 ml of a sample was taken to determine the initial virus titre. To inititate a virus inactivation experiment, 1 ml of a stock solution of chlorine (100 mg/l) was added to the virus suspension. At various times subsequently, 1 ml portions were removed and added to 1 ml of 2 × lactalbumin Hanks' balanced salt solution to neutralize the chlorine, and the remaining viruses were titred by plaque assay. The free available chlorine content of the stock solution was determined by an iodometric method, whereas the residual chlorine of the experimental solutions was determined by the diethyl-*p*-phenylenediamine colorimetric method. The final available chlorine concentration after 20 min was 0·3–0·4 mg/l, in comparison to the initial one which was 0·5 mg/l.

RESULTS

Effect of cyanuric acid on chlorine inactivation in CDF buffer

Fig. 1 shows the relationship between surviving virus and exposure time for coxsackievirus A24, enterovirus 70, and adenovirus 3 both with and without cyanuric acid. From the regression lines, it was estimated that the time required for 99·9% inactivation of coxsackievirus A24, enterovirus 70, and adenovirus 3 at 0·5 mg/l chlorine without cyanuric acid was 0·5, 0·12 and 0·14 min, while with 30 mg/l cyanuric acid it was 14·4, 2·5 and 2·1 min, respectively. Similar data were used to estimate the time required for 99·9% inactivation of all viruses studied. The time required for 99·9% inactivation of ten enteroviruses and two adenoviruses by 0·5 mg/l free available chlorine at pH 7·0 and 25 °C was prolonged approximately 4·8–28·8 times by the addition of 30 mg/l cyanuric acid to the system (Table 1).

Fig. 2 shows survival data for poliovirus 1 at various concentrations of available chlorine and cyanuric acid. The time required for 99·9% inactivation of the virus by 1·0 and 1·5 mg/l free available chlorine with 30 mg/l cyanuric acid was 3·5 and 2·1 min, the latter being longer than with 0·5 mg/l free available chlorine alone.

The time required for 99·9% inactivation by 0·5 mg/l free available chlorine with 1, 5 and 60 mg/l cyanuric acid was 2·0, 3·5 and 6·6 min (Fig. 3). This indicates that inactivation of the virus by chlorine was diminished by as little as 1 mg/l cyanuric acid and that the antagonism increased as the cyanuric acid concentration increased.

Effect of pH and temperature on chlorine inactivation with cyanuric acid

Table 2 gives the time required for 99·9% inactivation of poliovirus 1 with or without cyanuric acid at various pHs. A concentration of 30 mg/l cyanuric acid increased the time required for 99·9% inactivation of the virus by 0·5 mg/l free available chlorine at pH 6·0, 7·0 and 8·0 from 2·0, 0·8 and 1·2 min to 13·1, 5·6 and 5·7 min, respectively. Thus virus was inactivated more slowly at pH 6·0 than at

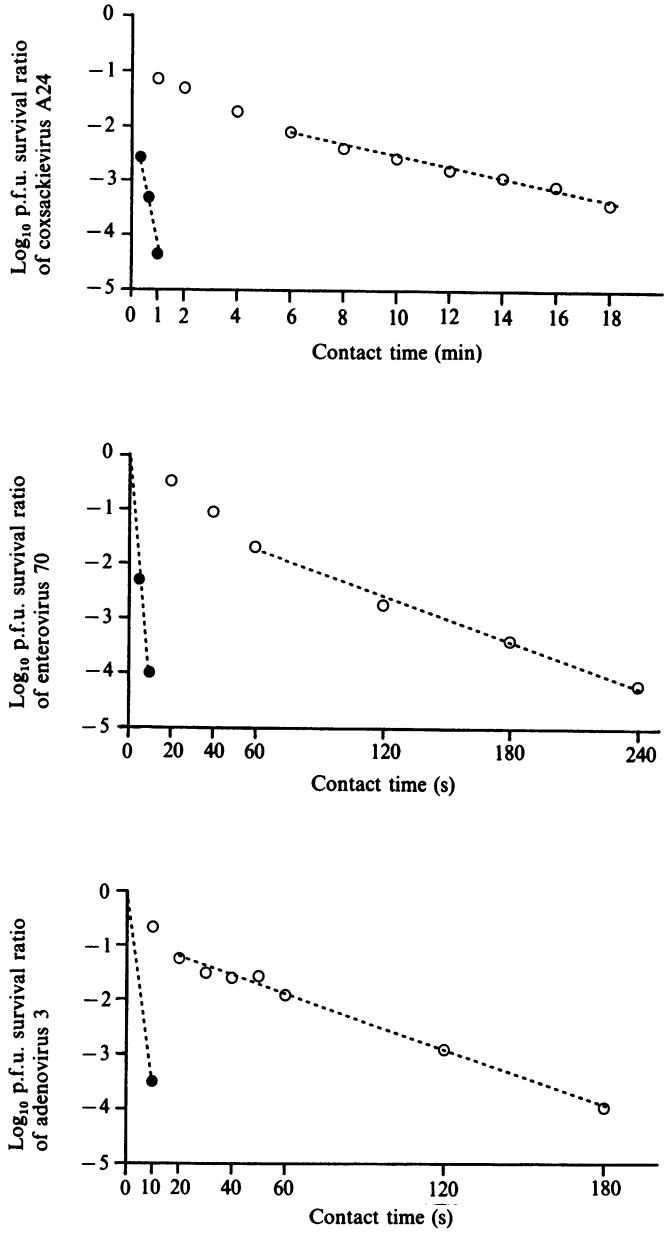

Fig. 1. Inactivation kinetics of coxsackievirus A24, enterovirus 70 and adenovirus 3 to 0·5 mg/l free available chlorine at pH 7·0, 25 °C, and cyanuric acid concentration of 0 mg/l (●) and 30 mg/l (○). – – – – –, Regression line.

pH 7·0 and 8·0 when cyanuric acid was added. Poliovirus aggregates at acid pH and becomes more resistant at pH 6·0 than at pH 7·0 and 8·0 (Jensen, Thomas & Sharp, 1980). These results suggest that the difference in degree of aggregation of the virion does not affect the inhibitory influence of cyanuric acid on the virucidal effect of chlorine.

A concentration of 30 mg/l cyanuric acid increased the time required for 99·9% inactivation of the virus by 0·5 mg/l free available chlorine at 20 and 30 °C from

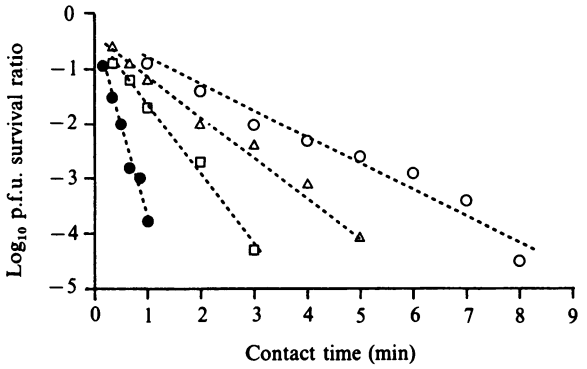

Fig. 2. Inactivation kinetics of poliovirus 1 at pH 7·0, 25 °C. Free available chlorine and cyanuric acid concentrations were 0·5 mg/l and 0 mg/l (●), 0·5 mg/l and 30 mg/l (○), 1·0 mg/l and 30 mg/l (△), and 1·5 mg/l and 30 mg/l (□), respectively. – – – –, Regression line.

Table 1. *Time required for 99·9% inactivation of various viruses by 0·5 mg/l free available chlorine with or without cyanuric acid (25 °C, pH 7·0)*

Virus	Time (min) for 99·9% inactivation at indicated cyanuric acid concentration (mg/l)		Ratio*
	0	30	
Polio 1 (Sabin)	0·8	5·6	7·0
Polio 2 (Sabin)	1·6	7·7	4·8
Coxsackie B3 (Nancy)	1·4	30·2	21·6
Coxsackie B4 (JVB)	1·1	10·4	9·5
Coxsackie B5 (Faulkner)	2·3	17·2	7·5
Echo 6 (D'Amori)	1·2	12·9	10·8
Echo 7 (Wallace)	0·6	4·8	8·0
Echo 11 (Gregory)	1·2	15·4	12·8
Coxsackie A24 (EH24/70)	0·5	14·4	28·8
Entero 70 (AHC(J670/71))	0·12	2·5	20·8
Adeno 3 (G.B.)	0·14	2·1	15·0
Adeno 7 (Gomen)	< 0·08	0·8	> 10·0

* Time required with 30 mg/l cyanuric acid/time required with 0 mg/l cyanuric acid.

Table 2. *Time required for 99·9% inactivation of poliovirus 1 at pH 6·0, 7·0 and 8·0 by 0·5 mg/l free available chlorine with or without cyanuric acid (25 °C)*

pH	Time (min) for 99·9% inactivation at indicated cyanuric acid concentration (mg/l)		Ratio*
	0	30	
6·0	2·4	13·1	5·6
7·0	0·8	5·6	7·3
8·0	1·2	5·7	4·8

* Time required with 30 mg/l cyanuric acid/time required with 0 mg/l cyanuric acid.

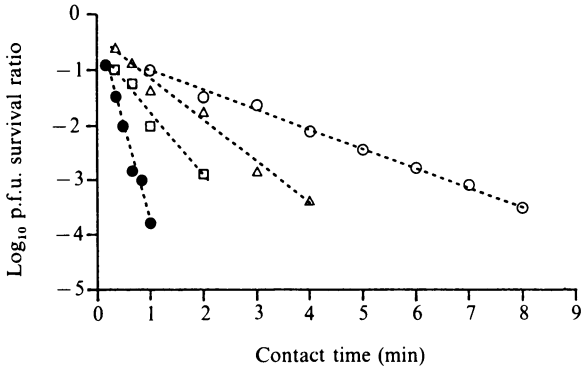

Fig. 3. Inactivation kinetics of poliovirus 1 to 0·5 mg/l free available chlorine at pH 7·0, 25 °C. Cyanuric acid concentration was 0 mg/l (●), 1 mg/l (□), 5 mg/l (△), and 60 mg/l (○). – – – – –, Regression line.

Table 3. *The effect of temperature on time required for 99·9% inactivation of poliovirus 1 by 0·5 mg/l free available chlorine with or without cyanuric acid (pH 7·0)*

	Time (min) for 99·9% inactivation at indicated cyanuric acid concentration (mg/l)		
Temperature	0	30	Ratio*
20	1.1	9.7	8.8
30	0.3	2.3	7.7

* Time required with 30 mg/l cyanuric acid/time required with 0 mg/l cyanuric acid.

Table 4. *Comparison of the quality of pool and tap water*

Water source	pH	Colour	Turbidity	NH_3-N (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)	Cl− (mg/l)	$KMnO_4$ demand (mg/l)
Pool water	6·8	2	0	0	0	0·36	45·5	8·5
Tap water	6·8	2	0	0	0	0·12	4·5	1·4

1·1 and 0·3 min without cyanuric acid to 9·7 and 2·3 min, respectively. The rates of inactivation were affected to the same extent by cyanuric acid at 20 and 30 °C. Consequently, the difference in the time required for 99·9% inactivation of the virus at 20 and 30 °C was increased from 0·8 min to 7·4 min with the addition of 30 mg/l cyanuric acid. It appears that a drop in water temperature decreases chlorine activity more markedly after the addition of cyanuric acid to swimming-pool water.

Effect of cyanuric acid on chlorine inactivation in swimming-pool and tap water

Table 4 summarizes the qualities of swimming-pool and tap water used in this study, and differences in nitrate nitrogen, chlorine chloride and potassium permanganate concentrations were found. Experiments were performed in these

Table 5. *Time required for 99·9% inactivation of poliovirus 1 in pool and tap water by 0·5 mg/l free available chlorine with or without cyanuric acid (25 °C)*

Water source	Time (min) for 99·9% inactivation at indicated cyanuric acid concentration (mg/l)		Ratio*
	0	30	
Pool water	1·9	11·2	5·9
Tap water	0·6	3·5	5·8

* Time required with 30 mg/l cyanuric acid/time required with 0 mg/l cyanuric acid.

waters at 25 °C with 0·5 mg/l free available chlorine, with and without 30 mg/l cyanuric acid, upon poliovirus 1. A concentration of 30 mg/l cyanuric acid increased the time required for 99·9% inactivation of the virus by 0·5 mg/l free available chlorine in pool and tap water from 1·9 and 0·6 min without cyanuric acid to 11·2 and 3·5 min, respectively (Table 5). The rates of inactivation were affected to the same extent by cyanuric acid in swimming pool and tap water. Consequently, the difference in the time required for 99·9% inactivation of the virus in pool and tap water was increased from 1·3 min to 7·7 min by the addition of 30 mg/l cyanuric acid. It appears that a drop in water quality reduces chlorine activity more markedly after the addition of cyanuric acid to swimming-pool water.

DISCUSSION

Our study reveals how cyanuric acid retards the disinfecting action of available chlorine on ten enteroviruses and two adenoviruses in CDF buffer. It is similar to the results of previous studies, which have shown that cyanuric acid reduces the bactericidal effect of available chlorine in distilled water (Andersen, 1965; Fitzgerald & DerVartanian, 1967; Robinton & Mood, 1967). However, another previous study using swimming-pool water indicates that cyanuric acid does not delay the bactericidal effect of chlorine (Kowalski & Hilton, 1966; Morgan, Gilcreas & Gubbins, 1966; Swatec, Raj & Kalbus, 1967). Our results show that the effect of cyanuric acid on the virucidal action of chlorine in swimming-pool water is similar to that in tap water and CDF buffer. It suggests that when cyanuric acid is used in swimming pools, a higher chlorine content is needed to obtain the same virucidal results as can be achieved under similar conditions when cyanuric acid is absent. Furthermore, with the addition of cyanuric acid, inactivation of poliovirus 1 and coxsackievirus B5, known to aggregate at acid and all pH values respectively (Jensen, Thomas & Sharp, 1980), is also slower than without it. It appears that aggregated viruses are more resistant to chlorine than dispersed ones (Young & Sharp, 1977), and the former are affected by cyanuric acid to the same degree as the latter. So far as can be determined, the risk of viral infection is greater in swimming-pool water with cyanuric acid when the chlorine content is raised to 0·4–1·0 mg/l. It should be pointed out, however, that in some areas with

intense sunlight available chlorine probably cannot be present without the stabilizing effect of cyanuric acid. The concern over chlorine levels which produce eye irritation is a common one, so the free available chlorine value should be increased to 1·5 mg/l when cyanuric acid is used in swimming pool water.

Temperature and water quality ranges in swimming pools usually play a role in the ability of chlorine to inactivate viruses. As the results of this study show, a drop in water temperature and quality reduce chlorine activity more markedly when cyanuric acid is present. The operator of the swimming pool using cyanuric acid should be required to pay special attention to water quality and temperature criteria.

Virus inactivation by chlorine was different for various viruses (Engelbrecht *et al.* 1980; Jensen, Thomas & Sharp, 1980; Payment, Tremblay & Trudel, 1985). In this study the influence of cyanuric acid on the virucidal effectiveness of chlorine also varied with the specific virus used. For example, poliovirus 2 was 99·9% inactivated in 1·6 min without cyanuric acid and in 7·7 min with 30 mg/l cyanuric acid, whereas coxsackievirus A24 was 99·9% inactivated in 0·5 min without cyanuric acid and in 14·4 min with 30 mg/l cyanuric acid. Coxsackievirus B5 was the most resistant to chlorine but was the second most resistant in the buffer with cyanuric acid. Coxsackievirus B3 was the third most resistant to chlorine but was the most resistant in the buffer with cyanuric acid. The wide range of influence of cyanuric acid on the virucidal effectiveness of chlorine displayed by different virus preparations and the similar influence in both a swimming pool and the 'clean' condition of buffered distilled water makes it necessary to investigate the influence of cyanuric acid in many test waters including organic contaminated waters and several swimming-pool waters.

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