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Inactivation of human rotavirus, SA11 and other enteric viruses in effluent by disinfectants

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SUMMARY

A preparation of infectious human rotavirus, isolated from faeces and resuspended in wastewater effluent, was shown to be inactivated by chlorine, chlorine dioxide, ozone and peracetic acid. Infectivity was assayed in MA 104 cells by the detection of cell-associated viral antigen by immunofluorescence. The inactivation curves were similar to those reported for other enteric viruses. Human rotavirus was at least as resistant as poliovirus, coxsackievirus, echovirus and f_2 coliphage and was strikingly less sensitive to inactivation than the simian rotavirus, SA 11. The latter was generally the most sensitive of the six tested viruses yet is often taken as being representative of the human rotaviruses.

INTRODUCTION

Human rotaviruses, like many other enteric viruses (Irving & Smith, 1981) may be present in large numbers in wastewater effluent (Smith & Gerba, 1982; Goddard, Bates & Butler, 1981). The health hazard this represents is difficult to determine, but there is no doubt that some enteric viruses such as human rotavirus, hepatitis A virus and Norwalk agent are pathogenic and well adapted to transmission via water. Therefore, the possibility that contact with effluents may result in enteric viral infection must be taken seriously, especially where untreated effluents are used for crop irrigation, are discharged into water used for recreation or contaminate water in which shellfish are cultivated.

So far as the consumption of shellfish is concerned, it is well established that gastroenteritis or hepatitis A may result, but only recently has satisfactory epidemiological evidence been obtained for the transmission of rotavirus and Norwalk agent to people who have swum in polluted sea water (Cabelli *et al.* 1982).

To minimize these health risks, care must be exercised over the procedures for disposal of sewage and effluents. For instance, effluents may be subjected to various treatments amongst which is disinfection, and several disinfectants are known to inactivate enteric viruses (Grabow, 1983; White, 1978), but most of the laboratory and field studies have been restricted to selected enteroviruses. Information about such key enteric viruses as human rotavirus was, until recently, unavailable

Table 1. *Effluent demand represented as disinfectant residual, determined as free and combined chlorine, 1 and 30 min after addition of selected doses*

Disinfectant	Initial dose (mg/l)	Residual 1 min		Residual 30 min	
		Free	Combined	Free	Combined
Chlorine	5·0	2·0	2·5	0·6	1·5
	10·0	6·5	7·2	4·5	5·0
	15·0	8·7	11·0	6·3	7·0
Chlorine dioxide	5·5	1·0*	—	0·8	—
	11·0	3·0	—	2·5	—
	13·8	4·0	—	3·0	—

* The method for chlorine dioxide estimation is based on a measure of free chlorine equivalent, therefore there is no 'combined chlorine' value.

because infectivity assays were impractical; however, an assay is now available (Banatvala *et al.* 1975) and has made possible the provision of the data reported herein.

MATERIALS AND METHODS

Viruses

Stock cultures of poliovirus type 1 (LSc 2ab), coxsackievirus B5 and echovirus 1 were prepared in BGM cell cultures and viral infectivity was assayed by the microtitre method in the same cells. The simian rotavirus, SA11, was cultivated in MA 104 cells in a serum-free medium containing trypsin, and infectivity was assayed by the plaque test in the same cells supported by a medium containing DEAE dextran and pancreatin (Smith *et al.* 1975). The human rotavirus, from a stool specimen, was assayed in MA 104 cells by detection of cell-associated viral antigen by indirect immunofluorescence (Banatvala *et al.* 1975). Bacteriophage f_2 was grown in *Escherichia coli* K_{12} Hfr and assayed by a soft agar lawn plate method (Balluz, Butler & Jones, 1978).

Disinfectants

Chlorine solution was obtained by bubbling the gas through chilled distilled water. Chlorine dioxide was prepared by heating to 80°C a mixture of powdered potassium chlorate and oxalic acid in a little water (Palin, 1948); the evolved gas was dissolved in chilled water. Peracetic acid was obtained as a 35% aqueous solution. Ozone was produced by an ozonator (Wallace and Tiernan). The liquid disinfectants, present in water or effluent, were assayed by the DPD method (Palin, 1979) and ozone by the colorimetric version of the DPD method (Palin, 1967). The DPD method for chlorine dioxide estimation gives a measure of the total chlorine equivalent, therefore in Table 1 there is no value against chlorine dioxide for 'combined chlorine'.

Inactivation experiments

Tests on the three liquid disinfectants (chlorine, chlorine dioxide and peracetic acid) were done in 500 ml Pyrex beakers mounted in a water bath and each provided with a stirrer motivated by an overhead drive. To each beaker 100 ml

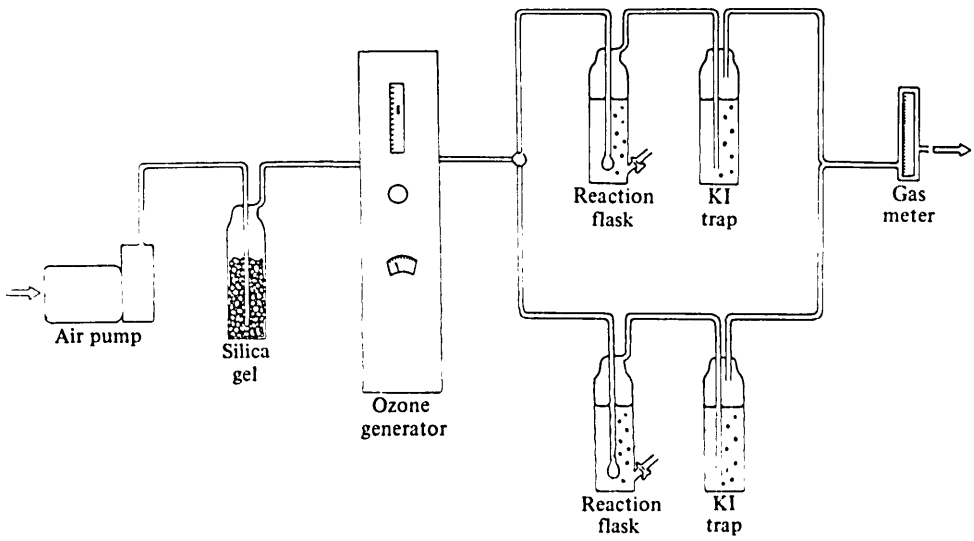

Fig. 1. Schematic diagram of the experimental apparatus for ozone (*KI* = potassium iodide).

of effluent was added followed by virus to provide approximately 10^5–10^6 infectious units/ml. Finally, the disinfectant (chlorine or chlorine dioxide) was added at a dose selected to provide a predetermined free and combined residual to take account of effluent demand. Typical values obtained in this work for chlorine and chlorine dioxide demand are given in Table 1. There was negligible demand for peracetic acid and demand for ozone was not determined, because it was pumped through Drechsel bottles (Fig. 1) containing 200 ml of effluent (seeded with virus) to provide a steady-state residual. After the samples had been collected the residual disinfectant was immediately neutralized with sodium thiosulphate and aliquots were stored at −20 °C until required for viral assay.

Effluents

All experiments were done using good-quality activated sludge effluent from batches which were stored at −12 °C until required. A slight alkaline shift in pH (from 7·2 to 7·8) was detected after the samples were thawed, but this was not considered important because pH values were always adjusted before each experiment. The other physical and chemical characteristics were unaltered (suspended solids 12·5 mg/l, ammonia 1·55 mg/l, biological oxygen demand 10·56 mg/l and chemical oxygen demand 37·22 mg/l).

RESULTS

A systematic examination of the sensitivity of human rotavirus to chlorine, chlorine dioxide, peracetic acid and ozone revealed that it was inactivated in a normal and predictable fashion (Fig. 2*a–d*). With each disinfectant a threshold concentration could be attained above which there was total inactivation within

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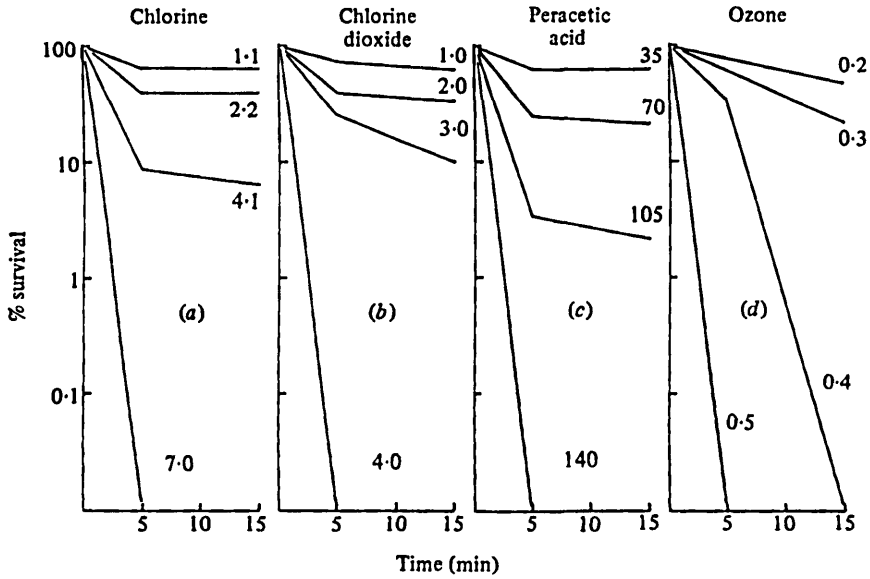

Fig. 2. Inactivation of human rotavirus in effluent (pH 7·2, 15 °C) by different disinfectants. Figures against each inactivation curve are concentrations of disinfectant in mg/l present at 30 min.

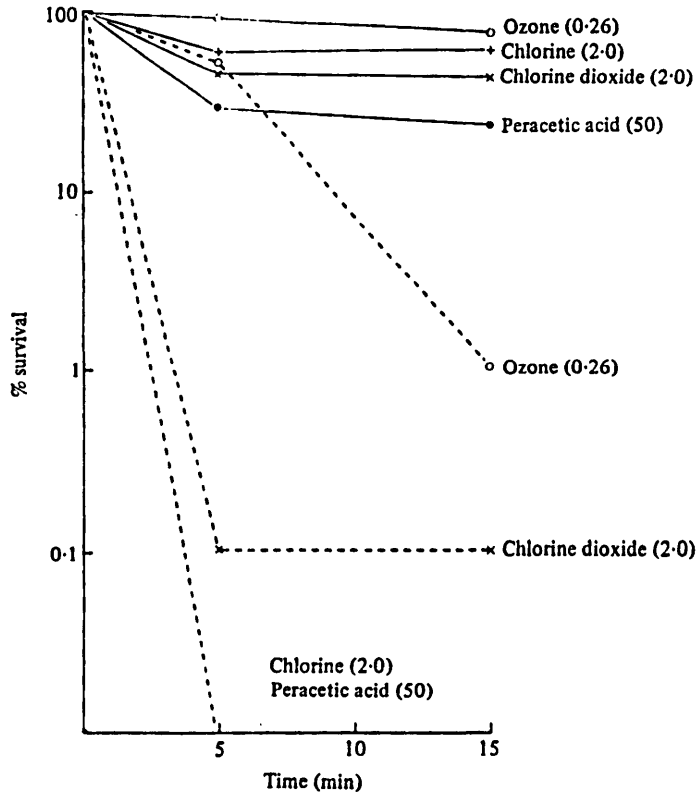

Fig. 3. Comparative sensitivity to different disinfectants of human rotavirus (solid lines) and SA 11 (dotted line) in effluent (pH 7·2, 15 °C). Figures (in brackets) against each disinfectant are concentration tested in mg/l present at 30 min.

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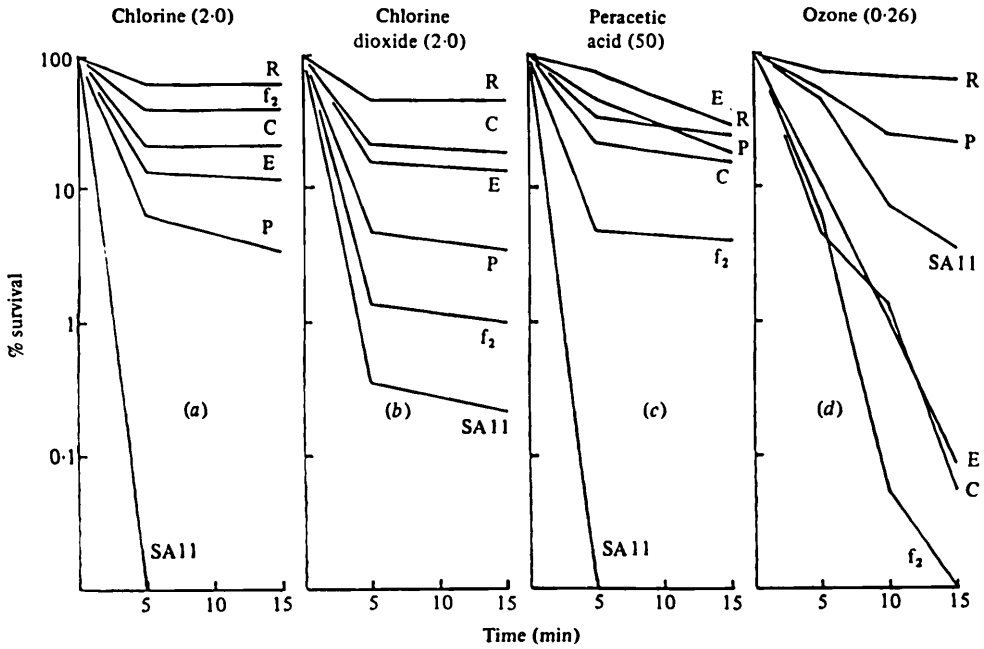

Fig. 4. Comparative sensitivity to different disinfectants of human rotavirus (R), simian rotavirus (SA 11), poliovirus (P), coxsackievirus (C), echovirus (E) and f_2 coliphage (f_2) in effluent (pH 7·2, 15 °C). Figures (in brackets) against each disinfectant are concentration tested in mg/l present at 30 min.

5 min, but at lower concentrations there was a biphasic reaction in the liquid disinfectants (chlorine, chlorine dioxide and peracetic acid) whereby most inactivation had occurred within five minutes. Thereafter little further loss of infectivity was observed despite the presence of residual disinfectant. Chlorine and chlorine dioxide were about equivalent in action on a molar basis, but much more peracetic acid was required for the same degree of inactivation.

In the presence of ozone, which unlike the liquid disinfectants was applied continuously, the reaction curves were different (Fig. 2*d*) particularly in that there was cumulative destruction of viral infectivity throughout the treatment.

The sensitivity of human rotavirus was compared to that of SA 11 by selecting a level of disinfectant just sufficient to inactivate low levels of the human strain, and under these conditions striking differences were observed (Fig. 3). Concentrations of chlorine and peracetic acid capable of inactivating at least 99·9% of SA 11 within 5 min permitted about 50% of the human rotavirus to survive. The differential observed with chlorine dioxide was almost as great, but in ozone SA 11 was only about 100-fold less sensitive than the human rotavirus.

When the sensitivity of the two rotavirus strains was compared with that of f_2 coliphage and the three enteroviruses (poliovirus, coxsackievirus and echovirus) it was noted that the human rotavirus was generally the most resistant whereas SA 11 was usually the most sensitive (Fig. 4*a–d*). The exception was in the presence of ozone, when f_2 coliphage was the most sensitive virus.

The order of sensitivity of the enteroviruses and f_2 coliphage was different for

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Table 2. *Rank order of sensitivity of enteric viruses to disinfection with various agents*

Disinfectant	Chlorine	Chlorine dioxide	Peracetic acid	Ozone
Virus type	Human rota	Human rota	Echo	Human rota
	f_2	Coxsackie	Human rota	Polio
	Coxsackie	Echo	Polio	SA11
	Echo	Polio	Coxsackie	Echo
	Polio	f_2	f_2	Coxsackie
	SA11	SA11	SA11	f_2

each disinfectant, for instance f_2 was the least sensitive to chlorine and the most sensitive to the other disinfectants (Table 2). The three enteroviruses also differed from each other with regard to resistance to the four disinfectants, with poliovirus being the most sensitive to chlorine and chlorine dioxide but least sensitive to ozone.

DISCUSSION

One of the most unexpected findings was that the two rotavirus types differed sharply in their sensitivity to disinfection. Thus the human rotavirus was more resistant than the simian rotavirus SA11 which, it should be noted, is often taken as representative of human rotavirus behaviour.

It was also interesting that the human rotavirus was also usually the most resistant of the tested viruses. The observation was not explained, but it is noteworthy that many endogenous viruses (Liu *et al.* 1971) and even endogenous bacteria (Aieta, Berg & Roberts, 1980) have been shown to be more resistant to disinfection than laboratory-adapted cultures of the same organism. In the present case it is just possible that the human rotavirus was protected in some way by residual faecal materials adsorbed to the virion surface or that the virus was present as aggregates of infectious particles. In the latter case a considerable degree of inactivation could occur without affecting the apparent infectivity, a phenomenon discussed by others (Floyd & Sharpe, 1977; Boardman & Sproul, 1977).

Also unexplained was the variation in sensitivity to different disinfectants shown by the various enteroviruses. However, none of this is necessarily surprising, because the mechanism of action of the disinfectants on different viruses may not be the same. Loss of viral infectivity may be through disruption of viral nucleic acids (O'Brien & Newman, 1979) or capsid proteins (Tenno, Fujioka & Loh, 1979) or both to varying degrees. The phenomenon of biphasic inactivation reported herein is characteristic of the response of viruses to such diverse factors as ultraviolet light and neutralizing antibody, as well as disinfectants under certain conditions. It has been widely discussed since the definitive paper by Chick (1908), but remains largely unexplained, and certainly there is no one unifying hypothesis.

What does emerge clearly from the work reported in this paper and similar studies is that the behaviour of one virus-type is unlikely to be exactly representative of another, so the concept of one indicator virus for water treatment is untenable, a view now becoming generally accepted (Morris & Waite, 1981).

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