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The relative resistance of f2 bacteriophage to inactivation by disinfectants

By MURRAY DRULAK, ALFRED M. WALLBANK and INGRID LEBTAG

The Department of Medical Microbiology and Infectious Diseases, University of Manitoba Medical College, Winnipeg, Manitoba, Canada R3E 0W3

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SUMMARY

f2 bacteriophage in the presence of fetal calf serum (at a final concentration of 10%) was exposed to six commonly used disinfectants for times of 10, 20 and 30 sec. At the end of exposure times skim milk neutralized the disinfectant activity and residual virus was assayed using the plaque technique. The 6 disinfectants considered were Javex, sodium hydroxide, ethanol, Wescodyne, One Stroke Ves-Phene and Sonacide. A 0·25% (w/v) solution of sodium hydroxide and 1/50 Javex (1200 parts/10^6 chlorine) were the most effective of the six disinfectants considered since 10^5 f2 bacteriophage were inactivated in 30 seconds in each instance. Since a 0·25% (w/v) solution of sodium hydroxide had a pH of 12·5 this made it too caustic to use as a disinfectant in many practical situations. It was concluded therefore that Javex at some dilution less than 1/50 (greater than 1200 parts/10^6 chlorine) was the most practical of the six disinfectants to use. Ethanol (95%, v/v) inactivated 10^3 f2 bacteriophage in 30 seconds while 1/20 Wescodyne and undiluted Sonacide inactivated 10^1 virus particles. Ves-Phene at a dilution of 1/50 was a completely ineffective virucide during the 30 sec exposure. The resistance of f2 bacteriophage to inactivation by these six disinfectants was compared with that of echovirus 11 and coxsackievirus B5. In all instances except exposure to undiluted Sonacide, f2 was comparable in resistance to inactivation and in many cases had greater resistance.

INTRODUCTION

Shah & McCamish (1972) have suggested that after treatment of waste-water with chlorine, the absence of f2 bacteriophage is a good indicator that enteroviruses in the water have not survived. The evidence is that f2 bacteriophage is more resistant to inactivation by combined chlorine than poliovirus. T2 bacteriophage is less suitable as such an indicator virus because it is more sensitive to chlorine than f2 bacteriophage. Oliveri *et al.* (1975) stated that f2 bacteriophage should not be used for evaluating the efficiency with which chlorine inactivated enteroviruses in water and waste-water systems since sufficient epidemiological and laboratory evidence was not available to support this. They proposed f2 bacteriophage be used as a classical model to obtain basic information concerning

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Table 1. *Disinfectants tested on f2 bacteriophage*

Disinfectant	Dilution tested
Sodium hydroxide	0·25% (w/v), pH 12·5
Wescodyne	1/20
Javex	1/100 (600 parts/10^6 chlorine)
	1/50 (1200 parts/10^6 chlorine)
One Stroke Ves-Phene	1/20
Sonacide	undiluted
Ethanol	95% (v/v)

fundamental parameters and reactions in disinfection of animal viruses, which are more difficult to work with. Hsu, Nomura & Kruse (1966) first reported the use of f2 bacteriophage as a model for disinfection studies with iodine. They showed that f2 bacteriophage RNA was resistant to iodination and that inactivation involved a reaction with viral protein. Such inactivation was inhibited by an increasing iodide ion concentration. This was also true for poliovirus 1.

The present work was done to determine the resistance of f2 bacteriophage to six selected disinfectants and to find out whether such resistance correlates well with that for enteroviruses.

MATERIALS AND METHODS

Micro-organisms

f2 bacteriophage. The original f2 stock from which pools of f2 bacteriophage were grown was obtained from Dr Norton Zinder, Rockefeller University, 66th St and York Avenue, New York. f2 bacteriophage pools were produced by the agar layer method of Swanstrom & Adams (1951), which was originally designed for the production of the T series bacteriophages.

Escherichia coli (*E. coli*) k37+. The *E. coli* k37+ used was obtained from Dr N. Zinder, Rockefeller University. A stock culture of *E. coli* k37+ was kept in cooked meat medium (Difco) at 4 °C. *E. coli* k37+ from such cultures was passaged three times on tryptone agar before being suspended in nutrient broth and used. The bacteria on the agar slants was 18 h old when suspended in nutrient broth.

Disinfectants

All disinfectants were diluted in sterile distilled water and prepared on the same day testing was done. The disinfectants considered in this study were the same as those in a previous study on echovirus 11 (Drulak, Wallbank & Lebtag, 1978*a*). Dilutions of the disinfectants tested are listed in Table 1.

Nutrient broth

Eight grams of nutrient broth powder (Difco) and 5 g of NaCl were added to 1 litre of distilled water. This was autoclaved at 121 °C for 30 min.

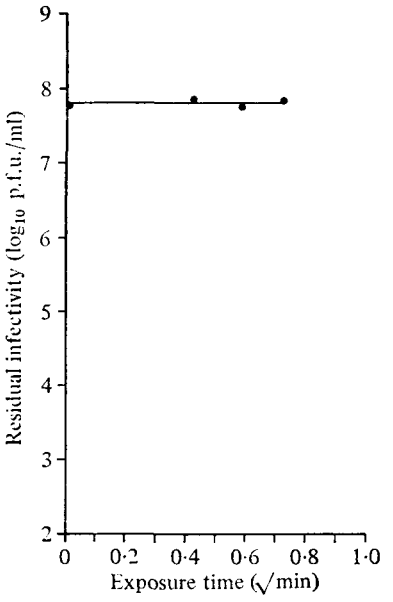

Fig. 1

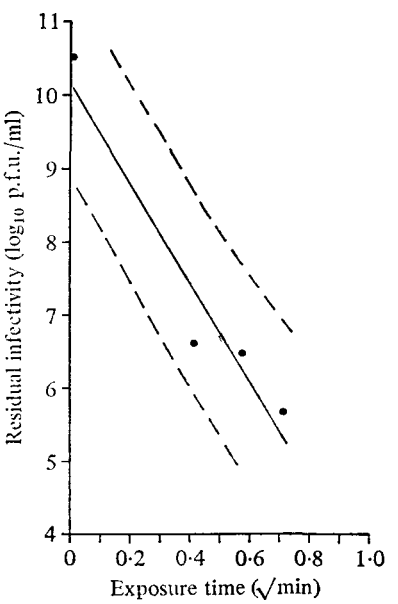

Fig. 2

Fig. 1. f2 bacteriophage in fetal calf serum exposed to 1/20 Ves-Phene. Residual virus versus square-root exposure time (min).

Fig. 2. f2 bacteriophage in fetal calf serum exposed to 0·25 % (w/v) sodium hydroxide. Residual virus versus square-root exposure time (min).

Tryptone agar

Tryptone overlay and plating agars were prepared by adding 7·5 and 15·0 g/l of Bacto-agar (Difco) respectively to Bacto-tryptone broth (Difco) as indicated by Loeb & Zinder (1961).

Plaque assay for f2 bacteriophage

Samples of 0·5 ml on which an assay was desired were added to a mixture of 0·02 ml *E. coli* k37+ and 2·5 ml of melted 0·75 % tryptone agar maintained in a water bath at 46 °C. Such mixtures were poured quickly over tryptone agar-containing petri dishes and allowed to harden. The plates were incubated for 24 hours at 37 °C and areas of bacterial lysis on the plates were readily observed, counted and expressed in plaque-forming units per ml (p.f.u./ml).

Disinfectant testing

The disinfectants were tested and neutralized controls were set up using the methods and reagents of Drulak *et al.* (1978*a*).

RESULTS AND DISCUSSION

f2 bacteriophage was exposed to dilutions of disinfectants listed in Table 1. Residual virus ($\log_{10}$ p.f.u./ml) was plotted against square-root minutes as discussed in Drulak *et al.* (1978*a*). Figs. 1 and 2 provide examples of the results: the

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Table 2. *Analysis of regression results for f2 bacteriophage (log_{10} p.f.u./ml) versus square-root exposure time (min)*

Disinfectant	Trial	r^2*	dF†	F
Undiluted Sonacide	1	0·931	1, 14	188·393
	2	0·876	1, 14	98·922
1/20 Wescodyne	1	0·836	1, 20	101·822
	2	0·811	1, 20	85·903
1/20 Ves-Phene	1	N.D.	N.D.	N.D.‡
	2	N.D.	N.D.	N.D.
95 % ethanol	1	0·894	1, 18	152·597
	2	0·882	1, 17	126·993
1/100 Javex	1	0·865	1, 25	160·321
	2	0·807	1, 14	58·658
1/50 Javex	1	0·946	1, 14	245·472
	2	0·943	1, 14	230·786
0·25 % NaOH	1	0·916	1, 14	152·004
	2	0·849	1, 14	78·948

* Correlation coefficient squared.
† Degrees of freedom for the F statistic.
‡ Not done, due to no significant viral inactivation occurring.

straight line in Fig. 2 was drawn using the calculated values of the linear regression as shown in Table 2. Each plotted point represents the mean of 4–8 replicate observations for residual virus. Table 2 shows that in all instances except one the F statistics and the correlation coefficient square (r^2) values were over 50 and 0·8 respectively. The high r^2 value and significant F value (at the 95 % confidence level) indicate linearity. The exception was the effect of 1/20 Ves-Phene and in this case there was no significant decrease in virus noted on exposure to the disinfectant. In fact, in this case as in many of the tests with ineffective virucides the residual virus values at 10, 20 and 30 sec were larger than the zero time values, probably because of deaggregation of the virus aggregates. Two experimental trials for each viral inactivation were done. In those inactivations where data were conducive to statistical analysis, the reproducibility of the inactivation relationship was determined by comparing the slopes of the different experimental trials statistically at the 95 % confidence level. The slopes of the two experimental trials were not significantly different at the 95 % confidence level: the data are supplied in Table 3.

The relative efficiency with which each disinfectant inactivated f2 bacteriophage was determined by comparison of slopes at the 95 % confidence level. Non-statistical comparisons had to be made when f2 bacteriophage exposed to 1/20 Ves-Phene was involved since the data were not conducive to statistical analysis as noted above.

Table 4 represents the results in such a fashion that the reader can see the relative efficiency of these disinfectants in inactivating f2 bacteriophage.

Conclusions of statistical and non-statistical comparisons are listed in Table 4.

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Table 3. *Reproducibility of log_{10} p.f.u./ml versus square root (min) relationship for f2 bacteriophage**

Disinfectant	Trial	*N*†	Square root (min) Mean	Square root (min) s.d.‡	Log 10 p.f.u. per ml Mean	Log 10 p.f.u. per ml s.d.‡	Slope	dF§	*F*
1/20 Wescodyne	1	22	0·462	0·249	2·93	0·350	−1·284	1, 40	2·082
	2	22	0·462	0·249	2·95	0·441	−1·594		
95 % ethanol	1	20	0·421	0·244	1·80	1·20	−4·646	1, 35	2·162
	2	19	0·446	0·261	2·27	1·08	−3·903		
Undiluted Sonacide	1	16	0·424	0·275	1·68	0·535	−1·878	1, 28	0·543
	2	16	0·424	0·275	1·55	0·504	−1·717		
1/100 Javex	1	27	0·487	0·235	2·20	0·513	−2·031	1, 39	0·222
	2	16	0·424	0·235	2·49	0·664	−2·171		
1/50 Javex	1	16	0·424	0·275	2·35	1·90	−6·735	1, 28	1·823
	2	16	0·424	0·275	2·86	1·69	−5·988		
0·25 % NaOH	1	16	0·424	0·275	2·30	1·95	−6·795	1, 28	1·374
	2	16	0·424	0·275	2·61	1·73	−5·805		
1/20 Ves-Phene	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.‖
	2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		

* Determined by comparison of slopes at the 95 % confidence level.
† Total number of experimental observations.
‡ Standard deviation.
§ Degrees of freedom.
‖ Not done, since data were not conducive to analysis of regression.

Table 4. *Relative resistance of f2 bacteriophage to the six disinfectants*

	Javex 1/50	Javex 1/100	NaOH 0·25 % (w/v)	undiluted Sonacide	Wescodyne 1/20	Ves Phene 1/20
1/100 Javex	–					
0·25 % (w/v) NaOH	*	+				
Undiluted Sonacide	–	*	–			
1/20 Wescodyne	–	–	–	*		
1/20 Ves-Phene	–	–	–	–	–	
95 % ethanol	–	+	–	+	+	+

– The disinfectant in the column at the left is less effective for inactivation of f2 bacteriophage than the disinfectant at the bottom of the table.

+ The disinfectant in the column at the left is more effective for inactivation of f2 bacteriophage than the disinfectant at the bottom of the table.

* The disinfectant in the column at the left is not different in effectiveness for inactivation of f2 bacteriophage from the disinfectant at the bottom of the table.

Although 95 % (v/v) ethanol has been considered the most effective disinfectant of the six tested in the past, since 10^6 virus particles were inactivated during a 20 sec exposure time in the case of echovirus 11 (Drulak *et al.* 1978*a*) and coxsackievirus B5 (Drulak *et al.* 1978*b*) this was not so for f2 bacteriophage. A 0·25 % (w/v) solution of sodium hydroxide (Fig. 2) and 1/50 Javex (1200 parts/10^6 chlorine) were the most effective of the six disinfectants considered since 10^5 virus particles were inactivated in 30 sec in each instance. A 0·25 % (w/v) solution of sodium hydroxide has a pH of 12·5 whch makes it too caustic to use in many practical situations. From this it can be concluded that Javex at some dilution less than 1/50 (greater than 1200 parts/10^6) is the most practical of the six disinfectants tested to use. Ethanol (95 %, v/v) is the third most effective disinfectant for it inactivated 10^3 f2 bacteriophage in 30 sec. Sonacide and Wescodyne are recommended for use by the manufacturer in undiluted form and at a dilution of 1/200 respectively. Undiluted Sonacide and 1/20 Wescodyne inactivated 10^1 f2 bacteriophage in 30 sec which demonstrates the relative inefficiency of these disinfectants as virucides. The results for f2 bacteriophage exposed to Wescodyne are similar to those of Wallbank *et al.* (1978) who found that 1/20 Wescodyne only inactivated $10^{1·5}$ poliovirus 1 in 80 min and 1/10 Wescodyne inactivated $10^{2·4}$ poliovirus 1 in 40 min. These tests were conducted in the presence of 8·5 % bovine serum albumin. These results for f2 bacteriophage exposed to Sonacide and Wescodyne also correlate well with those for echovirus 11 (Drulak *et al.* 1978*a*). f2 bacteriophage exposed to 1/20 Ves-Phene suffered no inactivation during a 30 sec exposure time (Fig. 1) which is comparable to the non-significant extent of inactivation incurred by echovirus 11 (Drulak *et al.* 1978*a*) and coxsackievirus B5 (Drulak *et al.* 1978*b*) exposed to 1/50 Ves-Phene.

Table 5. *f2 bacteriophage inactivation by different disinfectants relative to two enteroviruses*

	Coxsackievirus B5	Echovirus 11
1/100 Javex	–	*
1/50 Wescodyne	–	*/–
Undiluted Sonacide	+	+
0·25 % (w/v) sodium hydroxide	–	–
1/50 Ves-Phene	*	*
95 % ethanol	–	–

– The virus is less resistant to inactivation by disinfectant as compared to f2 bacteriophage.
+ The virus is more resistant to inactivation by disinfectant as compared to f2 bacteriophage.
* There is no difference in resistance to inactivation by disinfectant between the virus and f2 bacteriophage.

Table 6. *Comparison of neutralized controls with untreated controls for f2 bacteriophage experiments*

Disinfectant dilution	Trial	Untreated control*	Neutralized control*	Student's *t* test results†	Dilution of disinfectant in skim milk‡
1/100 Javex	1	2580, 1760	2480, 2760	No diff.	1/10
	2	2100, 2600	5400, 4900	No diff.	
1/50 Javex	1	1090, 950	1020, 950	No diff.	1/10
	2	1120, 1140	840, 1190	No diff.	
0·25 % NaOH	1	3600, 3000	3800, 2400	No diff.	1/10
	2	35200, 35800	25600, 32200	No diff.	
Undiluted Sonacide	1	35200, 25800	25600, 32800	No diff.	1/100
	2	18400, 16000	17600, 10400	No diff.	
1/20 Ves-Phene	1	16·2, 13·6	12·2, 12·2	No diff.	1/100
	2	4·0, 4·76	4·54, 5·96	No diff.	
1/20 Wescodyne	1	38·4, 42·0	49·0, 4·40	No diff.	1/100
	2	6·0, 6·2	9·6, 7·2	No diff.	
95 % Ethanol	1	3080, 2420	2420, 2720	No diff.	1/20
	2	4·0, 4·76	6·60, 5·56	No diff.	

* p.f.u./ml $\times 10^7$.
† Expressed at the 95 % confidence level unless otherwise specified (one tailed test).
‡ 17·5 % skim milk.

McCoy & Irwin (1974) conducted a study on inactivation by disinfectants of ØX 174, a single stranded DNA bacteriophage related to the parvoviruses. The test method used consisted of adding together 0·5 ml ØX 174 bacteriophage, 4·0 ml of Eagle's no. 2 medium containing 0·5 % bovine serum albumin and 0·5 ml of disinfectant at a concentration ten times greater than that which was to be tested. This was mixed and incubated for 10 min, after which residual ØX 174 bacteriophage was assayed. Results showed 1/10 sodium hypochlorite (4000–6000 parts/10^6 chlorine) reduced viral concentration by only 50 % while

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1/100 Wescodyne and 1/20 Wescodyne inactivated 10^1 and 10^6 ØX 174 bacteriophage respectively. The conclusions that may be drawn from the resistance of ØX 174 bacteriophage to inactivation by Wescodyne and chlorine are either that ØX 174 is basically more susceptible to inactivation by Wescodyne and less susceptible to inactivation by chlorine as compared to f2 bacteriophage or a difference in the parameters of the test system such as organic load and length of exposure time influenced the experimental results obtained.

The resistance of f2 bacteriophage to inactivation by these six disinfectants was compared with that for echovirus 11 (Drulak *et al.* 1978*a*) and coxsackievirus B5 (Drulak *et al.* 1978*b*). Mean slope data were compared at the 95% confidence level and non-statistical comparisons were made when data could not be statistically analysed. There were two basic types of comparisons from which conclusions in Table 5 were based. In the first type of comparison two viruses exposed to the same concentration of disinfectant had slopes compared. For example, echovirus 11 and f2 bacteriophage were both exposed to undiluted Sonacide. Since comparison of slopes for these two inactivations indicated they were significantly different and the slope for f2 bacteriophage was numerically greater, then it can be concluded that f2 bacteriophage is less resistant to inactivation by undiluted Sonacide as compared to echovirus 11. In the second type of comparison two viruses which were exposed to different concentrations of a disinfectant had inactivation curve slopes compared, when the virus exposed to the greater concentration had the smaller slope. In the context of this type of comparison echovirus 11 exposed to 1/50 Wescodyne and f2 bacteriophage exposed to 1/20 Wescodyne had slopes which were not significantly different. Since both viruses were not exposed to the same concentration of Wescodyne a multiple conclusion must be listed in Table 5. If f2 bacteriophage had been exposed to 1/50 Wescodyne either it would not have had a significantly different slope from that for echovirus 11 or the slope for f2 bacteriophage would have become numerically less due to exposure to now a lesser concentration of Wescodyne. This lesser slope for f2 bacteriophage would indicate it is more resistant to inactivation by 1/50 Wescodyne as compared to echovirus 11. In another instance coxsackievirus B5 exposed to 1/200 Wescodyne had a mean slope of $-7{\cdot}343$ and f2 bacteriophage exposed to 1/20 Wescodyne had a mean slope of $-1{\cdot}439$. Since the mean slopes were significantly different at the 95% confidence level then it can be concluded that coxsackievirus B5 was less resistant to inactivation by Wescodyne which is so indicated in Table 5. Table 5 demonstrates that in all comparisons between f2 bacteriophage and the two enteroviruses, with the exception of undiluted Sonacide, f2 bacteriophage is just as resistant to inactivation by disinfectant or more so, than coxsackievirus B5 and echovirus 11. Therefore, the possibility of using f2 bacteriophage as an indicator of resistance of enteroviruses to inactivation by disinfectant can be seriously considered. Further work must be done using a wide variety of virus–disinfectant combinations under different conditions before any firm conclusions can be drawn.

Table 6 lists the neutralized controls and untreated controls for each inactivation relationship as well as the dilution with 17·5% skim milk used for the neutrali-

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zation. A Student's t test comparison at the 95% confidence level between the untreated control and neutralized control indicated that the neutralized control did not have significantly less virus than the corresponding untreated control in all cases. These results indicate that 17·5% skim milk was an adequate neutralizer of the activity of a wide variety of disinfectants.

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