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The relative effectiveness of commonly used disinfectants in inactivation of coxsackievirus B5

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SUMMARY

Coxsackievirus B5 in the presence of fetal calf serum was exposed to six commonly used disinfectants for times of 10, 20 and 30 s. At the end of exposure times skim milk neutralized the disinfectant activity, with residual virus assayed by the plaque technique. The six disinfectants considered were Javex, sodium hydroxide, ethanol, Wescodyne, One Stroke Ves-Phene and Sonacide. Although 95% (v/v) ethanol was significantly more virucidal than dilutions of the other five disinfectants tested causing a 10^6 reduction in 20 s, it may not be practical to use in many instances. Next to 95 % (v/v) ethanol, 1/75 (800 parts/10^6) Javex, 0·25% (w/v) sodium hydroxide and 1/200 Wescodyne were the most effective virucides. These disinfectants were equal in effectiveness causing a 10^5 reduction of coxsackievirus B5 in 30 s. Of these three disinfectants Javex is the most practical to use since sodium hydroxide is caustic and Wescodyne is selective in its virucidal action. Undiluted Sonacide was a less effective virucide causing a less than 10-fold reduction of coxsackievirus B5 in 30 s. A 1/50 dilution of One Stroke Ves-Phene was the least effective virucide tested since it did not significantly inactivate coxsackievirus B5 in 30 s.

INTRODUCTION

Noll & Youngner (1959) observed that certain viruses especially those containing lipid combined with lipid. Such viruses were termed lipophilic. Viruses which did not combine with lipids were termed hydrophilic. While enveloped viruses such as influenza virus were termed lipophilic and viruses such as poliovirus, coxsackievirus and echovirus were termed hydrophilic, adenovirus which lacks an envelope reacted with lipid and was therefore classified as a lipophilic virus.

Klein & Deforest (1963) found that the resistance to inactivation of the hydrophilic viruses by some disinfectants was considerably greater than that of lipophilic viruses and vegetative bacteria. This resistance was ascribed to the failure of hydrophilic viruses to react with disinfectants having lipophilic properties such as long carbon chains. Lipophilic viruses were shown to be unusually susceptible to inactivation by disinfectants having lipophilic properties. No correlation between virus size or type of nucleic acid with disinfectant susceptibility was found.

Klein (1956) felt that the wide variation in susceptibility of viruses to inactivation by disinfectants precluded establishing a general figure of activity analogous to the phenol coefficient for bacteria.

The purpose of this article is to evaluate the virucidal activity of some commonly used disinfectants on a hydrophilic virus, coxsackievirus B5, using a test system that employs skim milk (SM) as a neutralizer of disinfectant activity, and a relatively short exposure time to the disinfectant. Such short exposure times were beneficial in differentiating between highly effective and ineffective disinfectants.

MATERIALS AND METHODS

R3 medium

R3 medium was prepared by the standard procedure (Drulak, Wallbank & Lebtag, 1978).

Virus

Coxsackievirus B5 was obtained from Dr Sattar and Dr Westwood of the University of Ottawa, Department of Microbiology. It was designated as 24-R5. The virus was passaged in BGM cells (African green monkey cell line) three times before use.

A pool of coxsackievirus B5 was prepared by the method used for echovirus 11 by Drulak *et al.* (1978).

Organic material

Fetal calf serum (Flow Laboratories, 936 W. Hyde Park Blvd, Inglewood, Calif. 90302) was used as added organic load in disinfectant testing. Fetal calf serum (FCS) was inactivated at 57 °C for 30 min and the same batch was used for all experiments.

Disinfectants

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

Temperature

Experiments were done in a laminar flow biological safety cabinet where the mean temperature was 26 °C. The temperature did not fluctuate more than 2 °C.

Minimal essential Medium (MEM) overlay for the plaque assay

Overlay was prepared as indicated by Drulak *et al.* (1978).

Table 1. *Disinfectants tested on coxsackievirus B5*

Disinfectant	Dilution tested
Sodium hydroxide	0·25 % (w/v), pH 12·5
Wescodyne	1/350, 1/200
Javex	1/100 (600 parts/10^6 chlorine)
	1/75 (800 parts/10^6 chlorine)
One Stroke Ves-Phene	1/50
Sonacide	Undiluted
Ethanol	95 % (v/v)

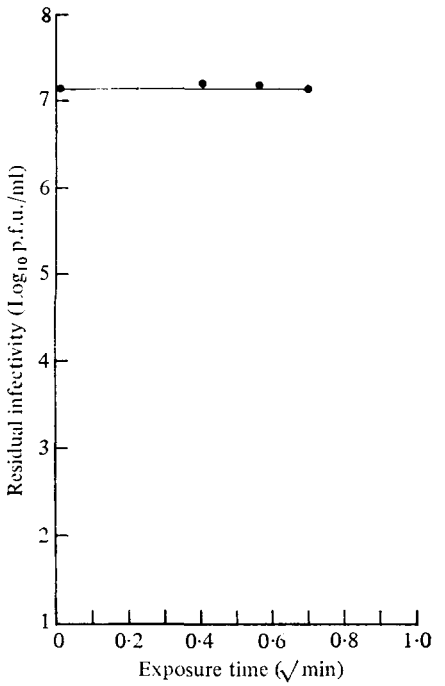

Fig. 1

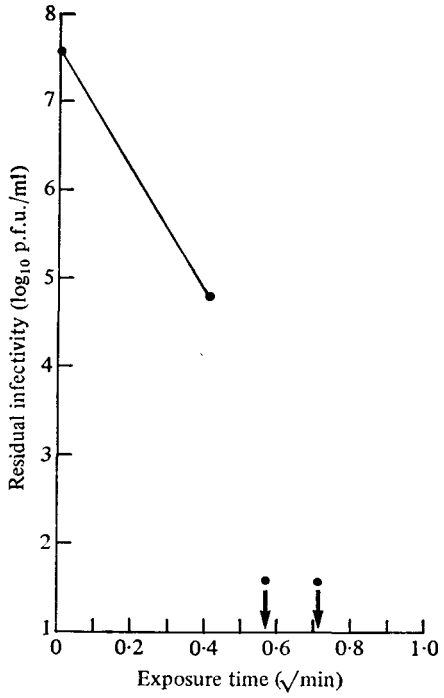

Fig. 2

Fig. 1. Coxsackievirus B5 in fetal calf serum exposed to 1/50 One Stroke Ves-Phene.
Fig. 2. Coxsackievirus B5 in fetal calf serum exposed to 95 % (v/v) ethanol.

Plaque assay for coxsackievirus B5

The plaque assay for coxsackievirus B5 was the same as that used for echovirus 11 by Drulak *et al.* (1978) except that the 37 °C incubation period before neutral red was added was 24 h instead of 48 h.

Disinfectant testing

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

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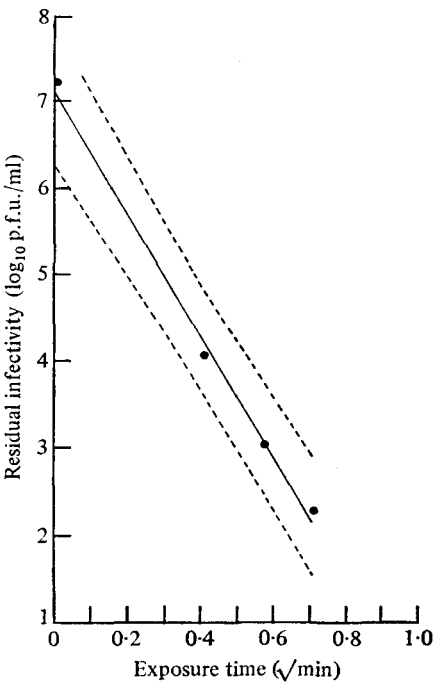

Fig. 3

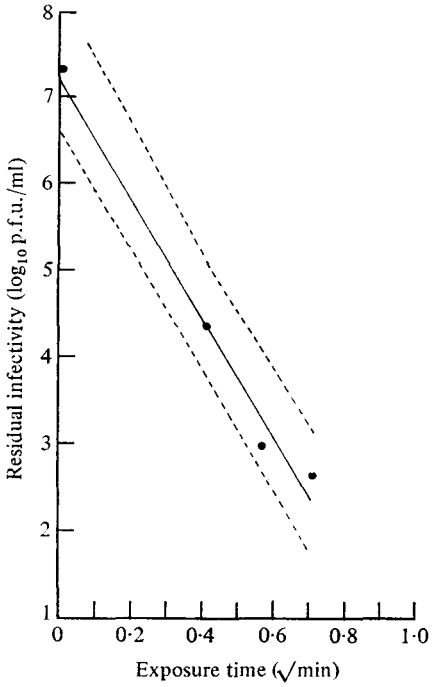

Fig. 4

Fig. 3. Coxsackievirus B5 in fetal calf serum exposed to 1/75 (800 parts/10^6 chlorine) Javex.

Fig. 4. Coxsackievirus B5 in fetal calf serum exposed to 0·25% (w/v) sodium hydroxide.

RESULTS AND DISCUSSION

Coxsackievirus B5 was exposed to dilutions of disinfectants listed in Table 1. In virus-disinfectant interactions residual virus versus exposure time was plotted as $\log_{10}$ p.f.u. versus square root min exposure time (Figs. 1–6). Each plotted point represented the mean of four to eight replicate observations for residual virus. Analysis of regression was done in all cases with the exception of those in which no significant decrease in virus was noted upon exposure to the disinfectant (e.g. Fig. 1) and in those cases in which disinfectant inactivation of the virus was so rapid that less than 3 numerical points were obtained (e.g. Fig. 2). Results in Table 2 consisted of an F statistic and the correlation coefficient squared (r^2) value. With one exception r^2 values were over 0·8 and the F values exceeded 50 and were therefore significant at the 95% confidence level. Such significant F values and large r^2 values indicate linear regression relationships.

The line in each regression relationship listed in Table 2 was based on the calculated values for the linear regression and was fitted around the actual mean data values.

Two experimental trials for each viral inactivation were done. In those instances where data were conducive to statistical analysis, the reproducibility of the inactivation relationship was determined by comparing the slopes of the different

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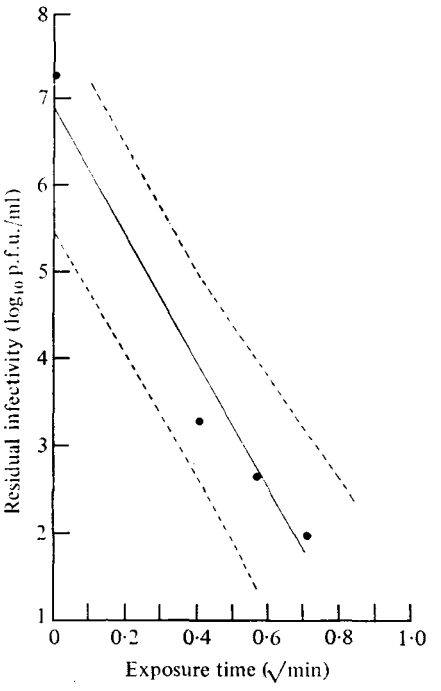

Fig. 5

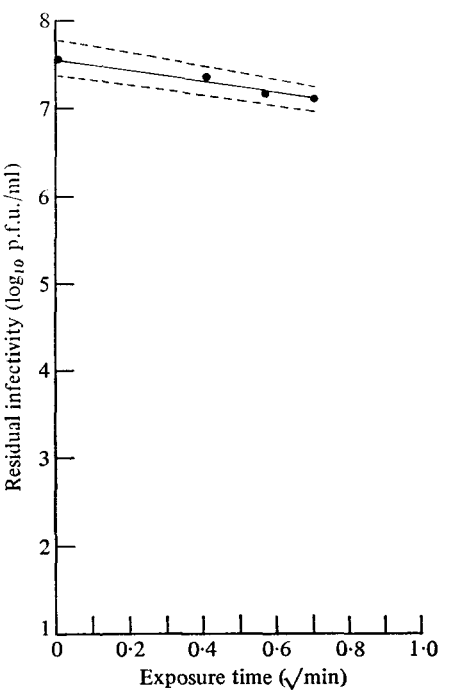

Fig. 6

Fig. 5. Coxsackievirus B5 in fetal calf serum exposed to 1/200 Wescodyne.

Fig. 6. Coxsackievirus B5 in fetal calf serum exposed to undiluted Sonacide.

experimental trials statistically at the 95% confidence level. In all instances in Table 3 the slopes of the two experimental trials were not significantly different at the 95% confidence level.

In instances where analysis of regression was done the relative efficiency with which each disinfectant inactivated coxsackievirus B5 was determined by comparison of slopes at the 95% confidence level. In comparisons where one or both of the residual virus versus exposure time relationships was not conducive to analysis of regression, non-statistical comparisons had to be made. For example, in Fig. 2 95% (v/v) ethanol caused a 10^6 reduction of coxsackievirus B5 in 20s whereas 1/75 (800 parts/10^6) Javex (Fig. 3) caused a 10^4 reduction of coxsackievirus B5 in 20 s, therefore 95% (v/v) ethanol was a more effective virucide than 1/75 (800 parts/10^6) Javex.

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

Although 95% (v/v) ethanol was the most effective virucide, causing a 10^6 reduction of coxsackievirus B5 in 20 s (Fig. 2) in this test system, ethanol must be used with caution since it cannot be diluted below 70% and retain virucidal activity. Therefore ethanol is unreliable to use unless in a large volume in comparison to the volume of virus-contaminated fluid to which ethanol is applied.

A 1/75 dilution (800 parts/10^6) of Javex (Fig. 3). 0·25% (w/v) sodium hydroxide (Fig. 4) and 1/200 Wescodyne (Fig. 5) were equally effective virucides and the

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Table 2. *Analysis of regression results for coxsackievirus B5 (log_{10} p.f.u./ml) versus square root min exposure time*

Disinfectant	Trial	r^2*	D.F.†	F
Undiluted Sonacide,	1	0·647	1, 23	42·216
	2	0·858	1, 16	96·366
NaOH, 0·25 % (w/v)	1	0·926	1, 17	211·341
	2	0·967	1, 20	582·474
Ves-Phene, 1/50	1	N.D.‡	N.D.	N.D.
	2	N.D.	N.D.	N.D.
Wescodyne, 1/350	1	0·950	1, 16	302·153
	2	0·844	1, 20	108·465
Wescodyne, 1/200	1	0·903	1, 17	157·885
	2	0·909	1, 20	199·667
Javex, 1/100	1	0·990	1, 23	2171·55
	2	0·964	1, 17	449·916
Javex, 1/75	1	0·967	1, 20	577·910
	2	0·974	1, 17	627·739
Ethanol 95 % (v/v)	1	N.D.§	N.D.	N.D.
	2	N.D.	N.D.	N.D.

* Correlation coefficient squared.
† Degrees of freedom for the F statistic.
‡ Not done, due to no significant viral inactivation occurring.
§ Not done, due to a rapid viral inactivation yielding a relationship of less than three numerical points.

most effective disinfectants next to 95 % ethanol. These disinfectants caused a 10^5 reduction of coxsackievirus B5 in 30 s.

Sodium hydroxide (0·25 %, w/v) had a pH of 12·5 making it too caustic to use as a virucide in some instances.

A dilution of 1/200 Wescodyne was the recommended use dilution cited by the manufacturer to be virucidal for poliovirus in a 5 min exposure time (West Chemical Products Inc, Long Island, N.Y.). Wallis *et al.* (1963) reported though that 10^4 p.f.u./ml of each of coxsackievirus A9 and B1, and echovirus 26 in tissue culture medium was not completely inactivated in 30 min upon exposure to 1/200 Wescodyne at 25 °C. It is interesting to note that although 1/200 Wescodyne was an effective virucide with reference to coxsackievirus B5, causing a 10^5 reduction in 30 s, even a dilution of 1/50 Wescodyne was ineffective against echovirus 11 (Drulak *et al.* 1978) causing only a 10-fold reduction of virus in 30 s. Since Wescodyne appeared to be selective in its virucidal action its use as a virucide should be restricted. On the other hand Javex appeared to be an effective virucide for both echovirus 11 and coxsackievirus B5 since 1/75 (800 parts/10^6) Javex caused a 10^5 reduction of coxsackievirus B5 in 30 s and 1/50 (1200 parts/10^6) Javex caused a $10^{3.5}$ reduction of echovirus 11 in 30 s (Drulak *et al.* 1978). On this basis dilutions of Javex less than 1/50 (1200 parts/10^6) are the most practical of the potential virucides considered. The actual dilution of Javex to use though should be dictated by each situation, with the amount of organic matter being of extreme importance.

Table 3. *Reproducibility of the log_{10} p.f.u./ml versus square root min relationship for coxsackievirus B5**

Disinfectant	Trial	*N*†	Square root min		Log_{10} p.f.u./ml		Slope	D.F.§	*F*
			Mean	S.D.‡	Mean	S.D.			
Ethanol, 95% (v/v)	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.	N.D.	
NaOH, 0·25% (w/v)	1	19	0·446	0·249	1·342	2·05	−7·565	1, 37	1·306
	2	22	0·462	0·249	2·079	1·75	−6·922		
Javex, 1/75	1	22	0·462	0·249	1·881	1·72	−6·691	1, 37	2·166
	2	19	0·446	0·261	2·462	1·65	−6·258		
Wescodyne, 1/200	1	19	0·446	0·261	1·631	2·01	−7·336	1, 37	0·021
	2	22	0·462	0·249	1·255	1·95	−7·449		
Javex, 1/100	1	25	0·474	0·239	2·582	1·05	−4·374	1, 40	2·648
	2	19	0·446	0·261	1·812	1·08	−4·073		
Wescodyne, 1/350	1	18	0·448	0·268	2·692	0·446	−1·621	1, 36	2·363
	2	22	0·462	0·249	2·111	0·532	−1·961		
Undiluted Sonacide	1	25	0·474	0·239	2·231	0·209	−0·703	1, 39	0·126
	2	18	0·448	0·268	2·279	0·190	−0·655		
One Stroke Ves-Phene, 1/50	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.	N.D.	

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

Table 4. *Relative effectiveness of the six disinfectants in inactivation of coxsackievirus B5*

Wescodyne 1/350	−						
Wescodyne 1/200	ϕ	+					
Javex 1/100	−	+	−				
Javex 1/75	ϕ	+	ϕ	+			
Ves-Phene 1/50	−	−	−	−	−		
Ethanol 95%	+	+	+	+	+	+	
Sonacide undiluted	−	−	−	−	−	+	−
	NaOH 0·25%	Wesco-dyne 1/350	Wesco-dyne 1/200	Javex 1/100	Javex 1/75	Ves-Phene 1/50	Ethanol 95%

−, Is less effective than; ϕ, is not different from; +, is more effective than.

Table 5. *Comparison of neutralized controls with titres for coxsackievirus B5 experiments*

Disinfectant dilution	Trial	Titre*	Neutralized controls*	Student's *t* test results†	Dilution of disinfectant in skim milk‡
Javex, 1/100	1	411,390	386,358	No difference	1/10
	2	396,390	302,344	No difference§	
Javex, 1/75	1	180,124	188,178	No difference	1/10
	2	284,252	260,270	No difference	
NaOH, 0·25% (w/v)	1	198,150	112,132	No difference	1/10
	2	316,248	326,290	No difference	
Undiluted Sonacide	1	270,276	386,378	No difference	1/100
	2	398,390	356,320	No difference§	
Wescodyne, 1/350	1	280,320	280,336	No difference	1/10
	2	94,114	114,146	No difference	
Wescodyne, 1/200	1	284,252	188,258	No difference	1/20
	2	180,124	200,146	No difference	
Ves-Phene, 1/50	1	198,150	180,192	No difference	1/20
	2	158,150	120,122	No difference‖	
Ethanol, 95% (v/v)	1	140,132	94,156	No difference	1/20
	2	372,342	320,346	No difference	

* p.f.u./ml × 10^5.
† Expressed at the 95% confidence level unless otherwise indicated (1 tailed test).
‡ 17·5% skim milk.
§ Significant at the 95% confidence level but not at the 99% level.
‖ Significant at the 99% confidence level but not at the 99·5% level.

Coxsackievirus B5 seemed to be quite susceptible to inactivation by Wescodyne since even a 1/350 dilution caused a 10-fold reduction of virus in 30 s.

The manufacturer recommends use of Sonacide in undiluted form for an exposure time of 10 min. Sonacide was an ineffective virucide causing a less than 10-fold reduction of coxsackievirus B5 in 30 s (Fig. 6) which made it less effective than even 1/350 Wescodyne. The active ingredient of Sonacide is 2% glutaral-

dehyde in the presence of a non-ionic surfactant at pH 3·5. Saitanu & Lund (1975) reported that although 2% glutaraldehyde caused a 100-fold reduction of coxsackievirus B5 in 1 min or less at pH 7·4 and 25 °C, the rate of inactivation was 10 times slower at pH 5.

A 1/50 dilution of One Stroke Ves-Phene was the least effective potential virucide tested since it did not significantly inactivate coxsackievirus B5 in 30 sec (Fig. 1). A dilution of 1/256 One Stroke Ves-Phene is the recommended use dilution.

The results obtained for Sonacide and One Stroke Ves-Phene in the inactivation of coxsackievirus B5 correlated well with those obtained for echovirus 11 (Drulak *et al.* 1978).

Table 5 lists the neutralized controls and titres for each inactivation relationship as well as the dilution with 17·5% SM used for the neutralization. A Student's *t* test comparison at the 95% confidence level between the titre and neutralized control indicated that the neutralized control did not have significantly less virus than the corresponding titre in 18 of 22 instances. In three of the remaining four cases, the neutralized control values were significantly less than the titre at the 95% confidence level but not at the 99% confidence level. In the remaining instance the neutralized control values were significantly less than the titre at the 99% confidence level but not at the 99·5% confidence level. In addition a Student's *t* test comparison between the titre and the cell susceptibility test control at the 95% level showed in each case that the cell susceptibility test control was not significantly less than the titre. These results indicate that 17·5% SM was an adequate neutralizer of the activity of a wide variety of disinfectants.

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