AN INVESTIGATION INTO THE VIRICIDAL ACTION OF TEEPOL, SODIUM HYDROXIDE, PROPYLENE GLYCOL AND DETTOL

By A. KLARENBEEK

Department of Bacteriology and Experimental Pathology of the Netherlands Institute for Preventive Medicine, Leiden, Holland

The viricidal action of various compounds (formaldehyde, chloramine, hexachlorophene, desogen, superol, mercuric chloride, furacine, and sodium hydroxide) on Columbia SK encephalomyelitis virus has been reported previously (Klarenbeek, 1951). The purpose of this investigation was to determine the suitability of these compounds as disinfectants in the virus laboratory. Naturally, the results are only decisive for the virus used, and for laboratory conditions. A separate study was devoted to the viricidal effect of ethylene oxide on Columbia SK and vaccinia virus (Klarenbeek & van Tongeren, 1954).

In the present investigation, Columbia SK virus was used. The starting dilution of virus (10^{-1}) was prepared from a 10% suspension of virulent mouse brain in distilled water, which was centrifuged for 15 min. at 3500 r.p.m. The LD_{50} titre was determined by intraperitoneal inoculation of mice with 0·1 ml. of serial tenfold dilutions. The tolerance of the compounds to be tested was determined also by intraperitoneal injection of mice. Virus and chemicals were mixed in varying concentrations, and kept in sterile tubes for varying periods at either 4° C. or −20° C. In addition, corresponding dilutions of virus without chemicals were treated similarly in order to check the activity of the virus under the experimental conditions. The mixtures, as well as the control virus suspensions, were inoculated intraperitoneally into groups of four (occasionally three, five, or six) mice.

Teepol. This detergent is an alkyl sulphate, which is used frequently as a synthetic wash-product, and for cleaning laboratory equipment, particularly glassware. There is, however, circumstantial evidence that the use of detergents may be hazardous. It became apparent that traces of insoluble calcium precipitates may be attached to the glass wall during the cleaning procedure with teepol and other anionic detergents. The precipitate may originate from a union of the detergent with calcium ions. It is the cause of trouble with prothrombin determinations (Lehmann, 1950, 1951). Moreover, difficulties have been met with in the use of culture tubes which had been cleaned with bactericidal detergents, e.g. desogen and zephirol. Inhibition of bacterial growth appeared to be due to traces of the detergent which had remained in the tubes (Dekking, 1952). Examination of the neutralization of the bacteriostatic action of compounds of the hexachlorophene group and of phenol derivatives by Tween-80 has led to the conclusion that the use of detergents in the laboratory may have incalculable consequences (Erlandson & Lawrence, 1953*a*, *b*). Finally, reports on the occurrence of arachnoiditis as a result of intraspinal injections with syringes and needles which had been cleaned

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with detergents have been published, and in dogs bilateral posterior paralysis has been produced experimentally by intraspinal injection with such an equipment (Winkelman, 1953; Paddison & Alpers, 1954).

The question arose, whether Teepol would interfere with the infectivity of viruses in tissues or suspension which are stored in glassware cleaned with the detergent.

Table 1. *Action of Teepol on Columbia SK-virus. Result of intraperitoneal inoculation of mice*

In vitro				*In vivo*			
Concentration of		Exposure		Concentration of		Inoculation dose	No. of mice dead/
Col. SK	Teepol	Time (days)	Temp. (° C.)	Col. SK	Teepol	i.p. (ml.)	inoculated
10^{-2}	10^{-2}	3	4	10^{-2}	10^{-2}	0·05	4/4
				10^{-3}	10^{-3}	0·05	4/4
				10^{-4}	10^{-4}	0·05	4/4
				10^{-5}	10^{-5}	0·05	4/4
				10^{-6}	10^{-6}	0·05	4/4
				10^{-7}	10^{-7}	0·05	4/4
				10^{-8}	10^{-8}	0·05	4/4
				10^{-9}	10^{-9}	0·05	4/4
10^{-2}	—	3	4	10^{-2}	—	0·05	4/4
				10^{-3}	—	0·05	4/4
				10^{-4}	—	0·05	4/4
				10^{-5}	—	0·05	4/4
				10^{-6}	—	0·05	4/4
				10^{-7}	—	0·05	4/4
				10^{-8}	—	0·05	4/4
				10^{-9}	—	0·05	4/4
10^{-2}	10^{-2}	7	−20	10^{-3}	10^{-3}	0·1	3/4
				10^{-4}	10^{-4}	0·1	4/4
				10^{-5}	10^{-5}	0·1	4/4
				10^{-6}	10^{-6}	0·1	4/4
				10^{-7}	10^{-7}	0·1	3/4
10^{-2}	—	7	−20	10^{-3}	—	0·1	4/4
				10^{-4}	—	0·1	4/4
				10^{-5}	—	0·1	4/4
				10^{-6}	—	0·1	3/4
				10^{-7}	—	0·1	4/4
10^{-2}	4×10^{-3}	30	−20	10^{-3}	4×10^{-4}	0·1	4/4
				10^{-5}	4×10^{-6}	0·1	4/4
				10^{-7}	4×10^{-8}	0·1	4/4
10^{-2}	—	30	−20	10^{-3}	—	0·1	4/4
				10^{-5}	—	0·1	4/4
				10^{-7}	—	0·1	4/4

In the experiments, doses of 0·05 and 1 ml. of a 1 % Teepol solution, which are far beyond the toxic doses, have been used. Table 1 shows, that Teepol in the concentrations used had no influence on the infectivity titre of Columbia SK virus. It is highly unlikely that Teepol, which might remain in far lower concentrations in glassware after thorough rinsing with water, exerts any viricidal action.

Sodium hydroxide. The viricidal action of sodium hydroxide on the virus of foot-and-mouth disease is well known. Vesicle fluid becomes avirulent within 5–10 sec. when treated with 2 % sodium hydroxide, within 30–40 sec. with a 1 % solution, and within 30–40 min. with a 0·5 % solution. It has been shown previously (Klarenbeek, 1951) that a 2·5 % suspension of Columbia SK virus in mouse brain is attenuated but not inactivated by exposure to 1 % sodium hydroxide for 20 min. Table 2 shows that complete inactivation is not obtained when a 1–2 % suspension of Columbia SK virus is exposed to 1–2 % sodium hydroxide for 24 hr. These results are of importance for the interpretation of the viricidal effect of hexachlorophene as reported earlier (Klarenbeek, 1951). This compound was dissolved

Table 2. *Action of sodium hydroxide on Columbia SK virus*

In vitro				*In vivo*			
Concentration of		Exposure		Concentration of		Inoculation dose	No. of mice dead/
Col. SK	NaOH	Time (hr.)	Temp. (° C.)	Col. SK	NaOH	i.p. (ml.)	inoculated
2×10^{-2}	10^{-2}	24	4	10^{-2}	Neutr.	0·05	4/4
				10^{-3}	Neutr.	0·05	4/4
				10^{-4}	Neutr.	0·05	4/4
				10^{-5}	Neutr.	0·05	1/4
				10^{-6}	Neutr.	0·05	0/4
				10^{-7}	Neutr.	0·05	1/4
				10^{-8}	Neutr.	0·05	1/4
10^{-2}	—	24	4	10^{-4}	—	0·05	4/4
				10^{-5}	—	0·05	4/4
				10^{-6}	—	0·05	4/4
				10^{-7}	—	0·05	4/4
				10^{-8}	—	0·05	5/5
4×10^{-2}	2×10^{-2}	6	4	2×10^{-2}	Neutr.	0·2	4/5
		24		2×10^{-2}	Neutr.	0·2	5/5
		24		2×10^{-5}	Neutr.	0·2	5/5
2×10^{-2}	10^{-2}	1	4	2×10^{-2}	Neutr.	0·2	5/5
		2	4	2×10^{-2}	Neutr.	0·2	5/5
		3	4	2×10^{-2}	Neutr.	0·2	5/5
		6	4	2×10^{-2}	Neutr.	0·2	5/5
		24	4	2×10^{-2}	Neutr.	0·2	5/5
		24	4	2×10^{-2}	Neutr.	0·2	5/5

in sodium hydroxide of a maximum concentration of 1 %. The influence of sodium hydroxide can, in the short periods of exposure, be neglected, so that the viricidal effect can be attributed to hexachlorophene.

Furacine and propylene glycol. Furacine has been shown to exert no influence on the infectivity of Columbia SK virus (Klarenbeek, 1951). Owing to the minute solubility of furacine in water, a concentration of only 0·047 % had been used. The present investigation was carried out with furacine dissolved in propylene glycol, in which a concentration of 0·2 % furacine was obtained.

Intraperitoneal injection of 0·2 ml of undiluted propylene glycol, and of the same amount of propylene glycol in which 0·2 % furacine had been dissolved, proved non-toxic for mice. When inoculating mice with a mixture consisting of

Table 3. *Action of propylene glycol with furacine on Columbia SK virus*

In vitro					*In vivo*				
Concentration of			Exposure		Concentration of				
Col. SK	Furacine (%)	Propylene glycol (%)	Time (hr.)	Temp. (° C.)	Col. SK	Furacine	Propylene glycol	Dose i.p. (ml.)	No. of mice dead/inocul.
10^{-2}	0·18	90	24	4	10^{-2}	Tenfold dilutions		0·1	4/4
			24		10^{-3}	Tenfold dilutions		0·1	3/4
			24		10^{-4}	Tenfold dilutions		0·1	2/4
			24		10^{-5}	Tenfold dilutions		0·1	4/4
			24		10^{-6}	Tenfold dilutions		0·1	4/4
			24		10^{-7}	Tenfold dilutions		0·1	3/4
10^{-2}	—	—	24	4	10^{-2}	—		0·1	4/4
					10^{-3}	—		0·1	4/4
					10^{-4}	—		0·1	4/4
					10^{-5}	—		0·1	4/4
					10^{-6}	—		0·1	4/4
					10^{-7}	—		0·1	4/4

Table 4. *Action of propylene glycol on Columbia SK virus*

In vitro				*In vivo*			
Concentration of		Exposure		Concentration of			
Col. SK.	Prop. glycol	Time (hr)	Temp. (° C.)	Col. SK.	Prop. glycol	Dose i.p. (ml.)	No. of mice dead/inoculated
10^{-2}	9×10^{-1}	24	4	10^{-3}	9×10^{-2}	0·1	1/6
				10^{-5}	9×10^{-4}	0·1	0/6
10^{-2}	—	24	4	10^{-5}	—	0·1	6/6
				10^{-7}	—	0·1	6/6
				10^{-9}	—	0·1	6/6
10^{-2}	9×10^{-1}	6	4	10^{-2}	9×10^{-1}	0·1	2/3
	9×10^{-4}	24	4	10^{-2}	9×10^{-1}	0·1	1/3
	9×10^{-2}	24	4	10^{-2}	9×10^{-2}	0·1	3/3
	9×10^{-3}	24	4	10^{-2}	9×10^{-3}	0·1	3/3
	—	24	4	10^{-2}	—	0·1	3/3
	—	24	4	10^{-8}	—	0·1	3/3

Table 5. *Action of Dettol on Columbia SK virus*

In vitro				*In vivo*			
Concentration of		Exposure		Concentration of			
Col. SK	Dettol	Time (hr.)	Temp. (° C.)	Col. SK	Dettol	Dose i.p. (ml.)	No. of mice dead/inoculated
10^{-2}	3×10^{-2}	1	4	10^{-3}	3×10^{-3}	0·1	4/4
10^{-2}	3×10^{-2}	3	4	10^{-3}	3×10^{-3}	0·1	4/4
10^{-2}	3×10^{-2}	5	4	10^{-3}	3×10^{-3}	0·1	4/4
		24	4	10^{-4}	3×10^{-4}	0·1	3/3
		24	4	10^{-6}	3×10^{-6}	0·1	2/3
		24	4	10^{-8}	3×10^{-8}	0·1	2/3
10^{-2}	—	24	4	10^{-4}	—	0·1	3/3
10^{-2}	—	24	4	10^{-6}	—	0·1	3/3
10^{-2}	—	24	4	10^{-8}	—	0·1	2/3

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1 part of a 10 % suspension of Columbia SK virus, and 9 parts of a 0·2 % solution of furacine in propylene glycol, no inactivation of the virus was observed. The final concentrations were 1 % of Columbia SK virus, 0·18 % furacine, and 90 % propylene glycol (Table 3). When, however, the same mixture but without furacine was used, the virus was inactivated almost completely by 90 % propylene glycol after exposure for 24 hr. No inactivation was obtained by 9 % and 0·9 % propylene glycol (Table 4).

Dettol. The bactericidal action of Dettol is due to the presence of a halogen derivative of xylenol (chloroxylenol) dissolved in aromatic oils and a neutral solution of soap. A 3 % solution of Dettol in water failed to inactivate a 1 % suspension of Columbia SK virus after exposure for 24 hr. at 4° C (Table 5).

SUMMARY

Teepol, sodium hydroxide, and Dettol failed to exert a viricidal effect on Columbia SK virus. The same virus was inactivated by 90 % propylene glycol after exposure for 24 hr. at 4° C. The viricidal effect of propylene glycol was neutralized by 0·2 % furacine.

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