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Inactivation of Viruses by Benzalkonium Chloride

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ABSTRACT

ARMSTRONG, J. A. (Sterling-Winthrop Research Institute, Rensselaer, N.Y.), AND E. J. FROELICH. Inactivation of viruses by benzalkonium chloride. Appl. Microbiol. **12**:132-137. 1964.—Benzalkonium chloride (as Roccal or Zephiran) was found to inactivate influenza, measles, canine distemper, rabies, fowl laryngotracheitis, vaccinia, Semliki Forest, feline pneumonitis, meningopneumonitis, and herpes simplex viruses after 10 min of exposure at 30 C or at room temperature. Poliovirus and encephalomyocarditis virus were not inactivated under the same conditions. It was concluded that all viruses tested were sensitive except members of the picorna group. The literature was reviewed.

The virucidal activity of quaternary ammonium compounds, in contrast to their antibacterial powers, has received little attention. Early work was reviewed by Lawrence (1950) and, more recently, Espmark and Salenstedt (1960) briefly reviewed some of the literature.

The study of the virucidal activity for 13 viruses of benzalkonium chloride USP, a mixture of alkylbenzyldimethyl ammonium chlorides (octyl through octadecyl), forms the basis of this report. Experiments reported here were performed for a variety of purposes and, therefore, are not completely uniform in their approach. However, in combination with the work of other investigators, already described in the literature, results described in this paper offer an improved image of the virucidal activity of benzalkonium chloride.

MATERIALS AND METHODS

Viruses. Viruses and strains used are shown in Table 1 together with details of their storage and assay. Tissue cultures used included the ATR and CATR lines of human amnion cells established in this laboratory. The 719 line of dog kidney cells, also established in this laboratory, as well as primary dog kidney cells, were used for the assay of canine hepatitis virus. The mice used were random-bred Swiss females. Embryonated hen's eggs were used for cultivation and assay of several viruses.

Virucidal procedures. Benzalkonium chloride USP solutions were prepared at suitable concentrations in distilled water, phosphate-buffered saline, 0.5% bovine albumin, fraction V in distilled water, or 5 or 10% normal rabbit serum in buffered saline. Experiments were carried out at room temperature or at 30 C in a water bath. In qualitative experiments, two or more virus dilutions were mixed with one or more dilutions of benzalkonium chloride, incubated for a suitable period (usually 10 min), rapidly diluted to avoid the inherent toxicity of benzalkonium chloride for cells, and injected into suitable hosts to determine the viability of the virus.

In quantitative experiments, a single concentration of virus suspension was used. Treatment with benzalkonium chloride and dilution were followed by titration of residual virus in the treated sample.

RESULTS

Picornaviruses. Poliovirus type 2 and encephalomyocarditis virus, tentatively assigned to the picornaviruses, proved to be entirely resistant to all concentrations of benzalkonium chloride tested (Table 2).

Myxo- and related viruses. Type A influenza virus was inactivated by concentrations of benzalkonium chloride as low as 0.025 mg/ml (Table 3). Measles and canine distemper viruses were also sensitive to the quaternary. The presence of serum in the reaction mixture protected influenza virus from the effect of 0.4 mg/ml of benzalkonium chloride. In some preliminary experiments, it was found that the presence of more than 5% chick embryo tissue would protect distemper virus.

Organisms of the psittacosis group. Feline pneumonitis and meningopneumonitis agents were inactivated by benzalkonium chloride after 10 min of exposure at room temperature (Table 4).

Other viruses. Rabies, fowl laryngotracheitis, Semliki Forest, and herpes simplex viruses were rapidly inactivated by low concentrations of benzalkonium chloride (Table 5). Vaccinia virus was inactivated by 1.33 mg/ml and, rather slowly, by 0.5 mg/ml but not by 0.1 mg/ml of quaternary. Infectious canine hepatitis virus was slowly inactivated by 1.33 mg/ml of benzalkonium chloride. After 10 min of exposure at 30 C, no effect was evident; however, after 2 hr, 99% inactivation occurred.

DISCUSSION

Table 6 summarizes some of the information in the literature, as well as data from the present study, on the inactivation of viruses by various quaternary ammonium compounds. It is worthwhile to consider the viruses group by group in evaluating the data, and to note the presence or absence of an activity pattern.

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Picorna viruses. Our data and all results in the literature indicate that picorna viruses are rather highly resistant to quaternary ammonium compounds. Cartwright and Thorne (1959) showed that foot-and-mouth disease virus is highly resistant to cetyltrimethylammonium bromide, but they performed no tests with benzalkonium chloride.

Adenoviruses. Although, under suitable conditions, adenoviruses are inactivated by quaternary ammonium compounds, our experience with infectious canine hepatitis virus suggests that the process is not rapid. When grown in primary dog kidney cells, infectious canine hepatitis virus was not inactivated by 1.33 mg/ml of benzalkonium chloride in 10 min, but some reduction of titer occurred when the virus was grown in serially passaged cells. Significant reduction in titer was evident after 120 min of exposure of the virus grown in either assay system (Table 7). Espmark and Salenstedt (1960) reported a reduction in the titer of adenovirus 7 of 1.25 to 1.5 log $TCID_{50}$ after 15 min of inactivation with 1 mg/ml of Colgon (a quaternary similar to benzalkonium chloride) at room temperature, but the details of their test procedures were not given. Klein and DeForest (1963) reported that the titer of adenovirus 2 was reduced 3 logs by 10 min of exposure to benzalkonium chloride at a concentration of 1:1,000 (w/v). There is some reason to suspect that canine hepatitis virus may be an unusually robust member of the adenovirus group; it is certainly a comparatively thermostable agent, and it may well be equally resistant to chemical inactivation.

Herpes simplex. The literature is in direct conflict on the inactivation of this virus by benzalkonium chloride. Describing the results of their experiments in which the virus was exposed to a quaternary at 10, 1, and 0.1 mg/ml for 15 min at room temperature, Espmark and Salenstedt (1960) stated, "No action was noted on . . . herpes simplex virus." Scott (1956), however, claimed that 10^6 EID_{50} are inactivated in 30 min at room temperature by 1 mg/ml of benzalkonium chloride. He noted that the action is inhibited by serum. Klein and DeForest (1963) also found the virus to be quaternary-sensitive; the work reported here supports their view.

Arbo viruses. Work with a number of arbo viruses at Fort Detrick (U.S. Army Chemical Corps Research and Development Command, 1962) showed them to be sensitive to benzalkonium chloride at the rather high concentration of 2%. We found Semliki Forest virus to be sensitive. Bucca (1956) showed that eastern equine encephalitis virus was inactivated by 1 mg/ml of quaternary.

Pox viruses. The results reported here, like those of other investigators (Klein, Kalter, and Mudd, 1945; Klein and DeForest, 1963), show that vaccinia virus may be inactivated by benzalkonium chloride. After 10 min of exposure to 1.33 mg/ml of the quaternary in a physiological medium containing some serum, at 30 C, the virus titer was reduced by 2.8 log units or 99.8%. Benzalkonium chloride (0.5 mg/ml) inactivated 98.3% of the virus under the same conditions. The Fort Detrick group (U.S. Army Chemical Corps Research and Development Command,

TABLE 1. *Virus strains and culture details*

Virus and strain	Storage	Assay
Poliovirus type 2 (MEF_1)	Mouse brain and cord at 4 C	Mouse, intracerebral
	Tissue culture fluid at −65 C	Tissue culture tube method; CATR cells
Encephalomyocarditis (MM)	Mouse brain at 4 C	Mouse, intraperitoneal
Influenza type A (PR_8 and Japan 305/57)	Allantoic fluid at −65 C	Egg, allantoic cavity, hemagglutination of allantoic fluids
Measles (Edmonston)	Tissue culture fluid at −65 C	Tissue culture tube method; CATR cells
Canine distemper (Onderstepoort)	Chorioallantoic membrane (50%) at −65 C	Egg, chorioallantoic membrane
Feline pneumonitis (No. 1)	Yolk sac (50%) at −65 C	Egg, yolk sac
Meningopneumonitis (Cal 10)	Mouse brain at −65 C	Mouse, intracerebral
Rabies (CVS and Flury LEP)	Mouse brain (20%) at −65 C	Mouse, intracerebral
Fowl laryngotracheitis (Lederle)	Chorioallantoic membrane (50%) at −65 C	Egg, chorioallantoic membrane
Semliki Forest	Mouse brain at 4 C	Mouse, intraperitoneal
	Tissue culture fluid at −65 C	Tissue culture tube method; ATR cells
Vaccinia (IHD)	Yolk sac (50%) at −65 C	Egg, yolk sac
	Tissue culture fluid at −65 C	Tissue culture tube method; CATR cells
Infectious canine hepatitis (Cornell)	Tissue culture fluid at −20 C	Tissue culture tube method; dog kidney cells
Herpes simplex (HF)	Tissue culture fluid at −65 C	Tissue culture tube method; ATR cells

1962) demonstrated the inactivation of variola virus by 2% quaternary.

Myxo- and related viruses. The etiological agents of influenza, measles, mumps, canine distemper, and Newcastle disease are readily inactivated by benzalkonium chloride. Inactivation of influenza virus in these tests occurred at concentrations as low as 0.025 mg/ml in 10 min. Espmark and Salenstedt (1960), Klein and DeForest (1963), and Knight and Stanley (1944) confirmed this observation. Measles and canine distemper viruses were shown in this laboratory to be sensitive to benzalkonium chloride. Tilley and Anderson (1947) were able to inactivate Newcastle disease virus with 0.1% cetyltrimethylammonium bromide.

Psittacosis-group organisms. Results reported here with the organisms of feline pneumonitis and meningopneumonitis, as well as Fort Detrick's (U.S. Army Chemical Corps Research and Development Command, 1962) report on psittacosis, show that this group of viruslike basophilic organisms are killed by benzalkonium chloride.

Other viruses. These studies show that fowl laryngotracheitis virus as well as rabies virus, which may have some morphological resemblance to the myxo-viruses, are killed by benzalkonium chloride. Dean, Baer, and Thompson (1963) and Wiktor and Koprowski (1963) described the local treatment of rabies-infected wounds with benzalkonium chloride, and confirmed its in vitro virucidal activity. Stock and Francis (1943) inactivated the virus

TABLE 2. *Picornaviruses not inactivated by benzalkonium chloride**

Virus and strain	Test system (observation)	Exposure		Highest concn tested
		Time	Temp	
		min	*C*	*mg/ml*
Poliovirus type 2 (MEF_1)	Mouse, intracranial (mortality)	10	RT	1.0
		120	RT	1.0
	T.C. CATR cells (cytopathic effect)	10	30	1.33
Encephalomyocarditis (M.M.)	Mouse, intraperitoneal (mortality)	10	RT	6.0

* Water was used as the diluent. RT = room temperature.

TABLE 3. *Inactivation of myxo- and related viruses by benzalkonium chloride*

Virus and strain	Test system (observation)	Diluent used	Exposure		Levels of activity (mg/ml)		
			Time	Temp*	Lowest effective	Doubtful	Highest ineffective
			min	*C*			
Influenza type A (PR8 or Japan 305/57)	Egg allantoic sac (hemagglutination titer)	PO_4 buffer	10	RT	0.025	—	—
			10	30	1.33†	—	—
		Serum (10%)	10	RT	—	—	0.4
					—	—	1.0
Measles (Edmonston)	Tissue culture CATR cells (cytopathic effect)	PO_4 buffer	10	30	1.33†	—	—
Canine distemper (Onderstepoort)	Egg chorioallantoic membrane (Pox on membrane)	Water	10	RT	0.1	—	—
			10	30	0.1	—	0.04
			30	30	0.1	—	0.032

* RT = room temperature.
† Single concentration quantitative test.

TABLE 4. *Inactivation of psittacosis group organisms by benzalkonium chloride*

Virus and strain	Test system (observation)	Diluent used	Exposure		Levels of activity (mg/ml)		
			Time	Temp*	Lowest effective	Doubtful	Highest ineffective
			min	*C*			
Feline pneumonitis (No. 1)	Egg, yolk sac (mortality)	Water	10	RT	0.1	—	0.04
		Serum (10%)	10	RT	0.2	0.1	—
		Bovine albumin, fraction V (0.5%)	10	RT	0.2	0.1	—
Meningo-pneumonitis (Cal 10)	Mouse, intracranial (mortality)	Water	10	RT	0.4	—	—

* RT = room temperature.

of lymphocytic choriomeningitis with approximately 0.36 mg/ml of benzalkonium chloride.

Effect of serum and other quaternary binding substances. Examination of Tables 1 through 4 shows that the presence of serum increases the lowest effective level of benzalkonium chloride required from 2-fold, in the case of feline pneumonitis, to greater than 16-fold, in the case of influenza. In preliminary tests with canine distemper virus, it was shown that concentrations of chick embryo tissues greater than 5% interfered with the inactivation process.

General considerations. Resistance of viruses to quaternary ammonium compounds seems to be confined to the picornavirus group. It is unfortunate that we possess no information on the reoviruses which, although larger, are of similar morphology. All ether-sensitive viruses (the arbo viruses, the herpes group and the myxo- and related viruses) tested to date were found to be sensitive to benzalkonium chloride. A number of ether-resistant viruses, including members of the poxvirus and adenovirus groups, are also sensitive to benzalkonium chloride.

Klein and DeForest (1963), noting the work of Noll and Youngner (1959) on the interactions of viruses with lipids, suggested that hydrophilic viruses, which are not adsorbed to lipids, will prove to be resistant to germicides having lipophilic properties. This hypothesis is in excellent agreement with the experimental observations, and it is possible that sensitivity to benzalkonium chloride may prove to be a simple method of determining the lipid affinities of viruses.

The results here reported, as well as those found in the literature, show all viruses tested except those belonging to the picornavirus group, to be killed by benzalkonium chloride. Thus, influenza, measles, rabies, psittacosis, herpes simplex, variola, vaccinia, yellow fever, eastern and Venezuelan equine encephalitides, Japanese B encephalitis, adenovirus type 7, canine distemper, infectious canine hepatitis, Newcastle disease, fowl laryngotracheitis, feline pneumonitis, meningopneumonitis, Semliki Forest, and lymphocytic choriomeningitis were shown to be susceptible to this quaternary. On the other hand, poliovirus, Coxsackie B 1, ECHO 6, ECHO 9, foot-and-mouth disease, encephalomyocarditis, and poliovirus muris (Theiler's virus) were found resistant to benzalkonium chloride. The virucidal activity of the quaternary ammonium compounds may be reduced in the presence of high concentrations of proteins or lipoids.

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TABLE 5. *Inactivation of miscellaneous viruses by benzalkonium chloride*

Virus and strain	Test system (observation)	Diluent used	Exposure		Levels of activity (mg/ml)		
			Time	Temp*	Lowest effective	Doubtful	Highest ineffective
			min	*C*			
Rabies (Flury or CVS)	Mouse, intracranial (mortality)	Serum (10%)	10	30	0.2	—	—
		Bovine albumin fraction V (0.5%)	10	30	0.4	0.2	—
					1.33†	—	—
		Water	10	30	1.0	—	0.32
Fowl laryngotracheitis (Lederle)	Egg, chorioallantoic membrane (Pox on membrane)	Water	10	RT	0.04	0.02	0.01
		Serum (10%)	10	RT	0.2	—	0.1
Semliki Forest	Mouse, intraperitoneal (mortality)	Water	2	RT	0.3	—	—
	Tissue culture ATR Cells (cytopathic effect)	Water	10	30	1.33†	—	—
Vaccinia (IHD)	Egg yolk sac (mortality)	PO_4 buffer	10	30	1.33†	—	—
	Tissue culture, CATR cells (cytopathic effect)	PO_4 buffer	10	30	1.33†	0.5	0.1
Infectious canine hepatitis	Tissue culture, dog kidney cells (cytopathic effect)	Water	10	30	—	—	1.33†
			120	30	1.33†	—	—
Herpes simplex (HF)	Tissue culture, ATR cells (cytopathic effect)	Water	10	30	1.33†	—	—

* RT = room temperature.

† Single concentration quantitative test.

TABLE 6. *Sensitivity of viruses to benzalkonium chloride or related quaternary ammonium compounds*

Virus	Reference*
Resistant	
Picornaviruses	
Poliovirus	TP; Espmark and Salenstedt (1960), Toomey and Takacs (1945), Klein and DeForest (1963)
Coxsackie B-1	Klein and DeForest (1963)
ECHO 6	Klein and DeForest (1963)
ECHO 9	Espmark and Salenstedt (1960)
Poliovirus muris (Theiler's)	Gönnert and Bock (1955)
Encephalomycarditis	TP, Schulz-Ehlbeck (1953)
Foot-and-mouth disease	Cartwright and Thorne (1959)
Sensitive	
Icosahedral DNA viruses	
Infectious canine hepatitis	TP, Espmark and Salenstedt (1960)
Herpes simplex	TP, Scott (1956), Klein and DeForest (1963)
Adenovirus, type 2	Klein and DeForest (1963)
Adenovirus, type 7	Espmark and Salenstedt (1960)
Arbo viruses	
Semliki Forest	TP
Yellow fever	U.S. Army Chemical Corps Research and Development Command (1962)
Venezuelan equine encephalitis	U.S. Army Chemical Corps Research and Development Command (1962)
Japanese B encephalitis	U.S. Army Chemical Corps Research and Development Command (1962)
Eastern equine encephalitis	Bucca (1956)
Pox viruses	
Vaccinia	TP, Espmark and Salenstedt (1960), Klein et al. (1945), Klein and DeForest (1963)
Variola	U.S. Army Chemical Corps Research and Development Command (1962)
Myxo- and related viruses	
Influenza	TP, Espmark and Salenstedt (1960), Knight and Stanley (1944), Klein and DeForest (1963)
Newcastle disease	Tilley and Anderson (1947)
Measles	TP
Canine distemper	TP, Celiker and Gillespie (1954)
Psittacosis-group organisms	
Psittacosis	U.S. Army Chemical Corps Research and Development Command (1962)
Feline pneumonitis	TP
Meningopneumonitis	TP
Miscellaneous viruses	
Rabies	TP, Dean et al. (1963), Wiktor and Koprowski (1963)
Fowl laryngotracheitis	TP
Lymphocytic choriomeningitis	Stock and Francis (1943)

* TP = data presented in this paper.

TABLE 7. *Inactivation of infectious canine hepatitis virus by benzalkonium chloride at 30 C*

Assay system	Expt no.	Diluent*	Virus titer (log $TCID_{50}$ per ml) after exposure for				Effectiveness at	
			10 min		120 min		10 min	120 min
			Infection control	BAC-treated†	Infection control	BAC-treated		
Primary dog kidney cultures	I	PBS	5.5	5.8	—	—	Ineffective	—
	III	Water	6.7	6.7	6.6	≤2.5	Ineffective	Effective
719 line of dog kidney cells	II	PBS	6.2	4.5	5.8	≤3.5	Slight	Effective
	III	Water	6.3	5.3	5.7	≤2.5	Slight	Effective

* Diluents: distilled water and Dulbecco's phosphate buffered saline (PBS).
† BAC = benzalkonium chloride.

LITERATURE CITED

BUCCA, M. A. 1956. The effect of various chemical agents on eastern equine encephalomyelitis virus. J. Bacteriol. **71**:491–492.

CARTWRIGHT, S. F., AND H. V. THORNE. 1959. Some applications of detergents to the study of the virus of foot-and-mouth disease. J. Gen. Microbiol. **20**:61–77.

CELIKER, A., AND J. H. GILLESPIE. 1954. The effect of temperature, pH, and certain chemicals on egg-cultivated distemper virus. Cornell Vet. **44**:276–280.

DEAN, D. J., G. M. BAER, AND W. R. THOMPSON. 1963. Studies on the local treatment of rabies-infected wounds. Bull. World Health Organ. **28**:477–486.

ESPMARK, A., AND C. R. SALENSTEDT. 1960. Virus och invertsåpor. Nord. Med. **64**:1194–1196.

GÖNNERT, R., AND M. BOCK. 1955. Zur Resistenz von Viren. Z. Hyg. Infektionskrankh. **141**:60–63.

KLEIN, M., AND A. DEFOREST. 1963. The inactivation of viruses by germicides. Chem. Specialties Mfrs. Assoc. Proc. Mid-Year Meeting **49**:116–118.

KLEIN, M., S. S. KALTER, AND S. MUDD. 1945. The action of synthetic detergents upon certain strains of bacteriophage and virus. J. Immunol. **51**:389–396.

KNIGHT, C. A., AND W. M. STANLEY. 1944. The effect of some chemicals on purified influenza virus. J. Exptl. Med. **69**:291–300.

LAWRENCE, C. A. 1950. Surface-active quaternary ammonium germicides. Academic Press, Inc., New York.

NOLL, H., AND J. S. YOUNGNER. 1959. Virus-lipid interactions. II. The mechanism of adsorption of lipophilic viruses to water-insoluble polar lipids. Virology **8**:319–343.

SCHULZ-EHLBECK, H. W. 1953. Beitrag zur Frage der Virusinaktivierung. Klin. Wochschr. **31**:527.

SCOTT, T. F. McN. 1956. Herpes simplex, p. 313–340. *In* Diagnostic procedures for virus and rickettsial diseases. American Public Health Association, New York.

STOCK, C. C., AND T. FRANCIS. 1943. The inactivation of the virus of lymphocytic choriomeningitis by soaps. J. Exptl. Med. **77**:323–336.

TILLEY, F. W., AND W. A. ANDERSON. 1947. Germicidal action of certain chemicals on the virus of Newcastle disease. Vet. Med. **42**:229–230.

TOOMEY, J. A., AND W. S. TAKACS. 1945. Effect of cationic detergents in cotton rats: neutralizing effect of cetamium against poliomyelitis virus. Arch. Pediat. **62**:337–339.

U.S. ARMY CHEMICAL CORPS RESEARCH AND DEVELOPMENT COMMAND. 1962. Practical procedures for microbial decontamination. Technical manuscript no. 2, p. 29. Armed Services Technical Information Agency. Arlington, Va.

WIKTOR, T. J., AND H. KOPROWSKI. 1963. Action locale de certain médicaments sur l'infection rabique de la souris. Bull. World Health Organ. **28**:487–494.