Inactivation of Vesicular Stomatitis Virus by Disinfectants

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Twenty-four chemical disinfectants considered to be viricidal were tested. Ten disinfectants were not viricidal for vesicular stomatitis virus within 10 min at 20°C when an LD₉₀ titer of 10⁴.₅ virus units per 0.1 ml were to be inactivated. Quantitative inactivation experiments were done with acid, alkaline, and a substituted phenolic disinfectant to determine the kinetics of the virus inactivation. Substituted phenolic disinfectants, halogens, and cresylic and hydrochloric acids were viricidal. Basic compounds such as lye and sodium metasilicate were not viricidal.

The virical activity of disinfectants has received much less attention than their activity as bactericides. Disinfectants have been reported to be viricidal under certain conditions (1, 3, 6; M. Klein and A. DeForest, Fed. Proc., p. 319, 1965). The virucidal activity of disinfectants has been demonstrated by the author previously (8) as a method for testing viricides against vesicular stomatitis virus (VSV) similar to those employed for bactericide testing (2) and was used to determine the virical activity of 24 disinfectants.

Experiments were also carried out to determine the rate of virus inactivation with 10% sodium hydroxide, 5% acetic acid, and a substituted phenolic disinfectant.

MATERIALS AND METHODS

The techniques used to obtain these data were the same as described previously (8) with the following exceptions.

Disinfectant tests. Virus-disinfectant contact times were 5 and 10 min. The 30-min interval was eliminated since 10 min has been established as a maximal practical exposure time for effective viricides in animal disease control work.

Speed of inactivation. Experiments were completed with seven time intervals. These were 0.5, 1, 2, 3, 4, 7.5, and 10 min. Additional time intervals of 30 and 120 min and 24 hr were used for NaOH. The disinfectant was diluted at the end of each time interval by the addition of 9 ml of phosphate-buffered saline (PBS) to the disinfectant-virus mixture, and, where acid or base was used, sterile 0.1 N NaOH or 0.1 N HCl was added to neutralize the pH of the PBS.

Quantitative determinations of virus titer were measured by using serial 10-fold dilutions of virus-disinfectant mixture in embryonating chicken eggs for each time interval. The titer of virus was estimated by the LD₉₀ method of Reed and Muench (7).

Disinfectants. The 24 disinfectants are listed in Table 1.

RESULTS

Of 24 disinfectants, 10 were not viricidal with the previously described procedure. Alkaline chemicals tested were not viricidal for VSV, e.g., VSV survived for 24 hr in 10% NaOH (pH 12.2). Virulent virus was demonstrated after treatment with 10% KOH (pH 13.3), 10% Na₂CO₃ (pH 11.1), and 5% Na₂SiO₃ (pH 12.1) for 10 min. The quaternary ammonium compound, disinfectant G, was not viricidal at concentrations of 0.1 to 5.0%. In addition, sulfuric acid, acetic acid, isopropanol, ethyl alcohol, and Formalin were not viricidal at concentrations tested. The results are listed in Table 2 with the range of concentrations. Identical results were obtained for at least four replications of each disinfectant. The 14 other disinfectants were viricidal, but in some cases at concentrations greater than suggested by the manufacturer. The minimum viricidal concentration and the range of concentrations tested are listed in Table 3. Consistent results were not obtained with disinfectant E. The manufacturer's lot 1 was viricidal, whereas a second lot was not viricidal at the same concentration.

The speed of inactivation was determined with NaOH, acetic acid, and disinfectant L. The death curve with NaOH and acetic acid was not linear, being initially rapid, followed by a decrease in rate. Virulent virus was not detectable after 30 min with 5% acetic acid, whereas, with 10% NaOH, it was still virulent after 24 hr.

Disinfectant L at 2% concentration was rapidly viricidal and virulent virus was not detectable after 2 min. The virus survived 0.5%
### Table 1. Active ingredients in commercial disinfectants tested

| Disinfectant | Active ingredients                                                                 | Concentration | Lots tested |
|--------------|------------------------------------------------------------------------------------|---------------|-------------|
| A            | Mixed phenols as saponified cresol solution                                        | 30-53         | 1           |
| B            | Mixed phenols as saponified cresol solution                                        | 30-53         | 1           |
| C            | Mixed phenols as saponified cresol solution                                        | 30-53         | 1           |
| D            | o-Phenylphenol, sodium salt                                                        | 100.0         | 1           |
| E            | o-Phenylphenol                                                                     | 15.0          | 1           |
| F            | p-tetra-Amylphenol                                                                  | 6.3           | 2           |
| G            | Alcohol                                                                            | 4.7           |             |
| H            | Alkyl dimethylbenzylammonium chlorides (benzalkonium chloride)                     | 10            | 1           |
| I            | o-Benzyl p-chlorophenol                                                             | 2.5           |             |
| J            | p-Tertiary amyl phenol                                                              | 2.3           |             |
| K            | o-Phenylphenol                                                                      | 1.3           |             |
| L            | 2,2',Methylenebis (3,4,6-trichlorophenol)                                           | 0.2           |             |
| M            | o-Benzyl p-chlorophenol                                                             | 5.0           |             |
| N            | p-Tertiary amyl phenol                                                              | 4.5           |             |
| O            | o-Phenylphenol                                                                      | 2.6           |             |
| P            | 2,2',Methylenebis (3,4,6-trichlorophenol)                                           | 0.4           |             |
| Q            | Isopropanol, sodium dodecylbenzene sulfonate, sodium o-benzyl p-chlorophenate      | 18.2, by weight |             |
| R            | sodium xylene sulfonate, sodium p-tertiary amyl phenate, sodium 4-chloro-2-phenyl | 62.5          |             |
| S            | phenate, sodium 2-chloro-4-phenyl phenate, tetrasodium ethylenediaminetetraacetate, |              | 1           |
|              | sodium 6-chloro-2-phenyl phenate                                                    |               |             |
| T            | o-Benzyl p-chlorophenol                                                             | 6.4           |             |
| U            | p-Tertiary amyl phenol                                                              | 5.8           |             |
| V            | o-Phenylphenol                                                                      | 3.3           | 1           |
| W            | 2,2',Methylenebis (3,4,6-trichlorophenol)                                           | 0.4           |             |
| X            | Polyethylene glycol polypropoxy polyethoxy ethyl alcohol-iodine complex            | 7.9 (w/w)     |             |
| Y            | Nonyl phenoxypolyethoxy ethyl alcohol-iodine complex                                | 7.6 (w/w)     |             |
| Z            | Hydrogen chloride (provides 1.6% available iodine)                                  | 0.10 (w/w)    |             |

- **Acetic acid**
- **HCl**
- **H₂SO₄**
- **Na₂SiO₃**
- **KOH**
- **NaOH**
- **Na₂CO₃**
- **Formalin**
- **Ethyl alcohol**
- **Isopropanol**
- **Phenol**
- **NaOCl**

* Concentrations of active ingredients as stated by the manufacturer.
* C₁₂, C₁₄, C₁₆, and other related alkyl groups from C₈ to C₁₈.
* Analytical reagent grade.
* National Formulary.

Disinfectant L for more than 10 min but less than 20 min. The survival curves are represented in Figs. 1 and 2.

**DISCUSSION**

The virus dilution test was employed to determine the viricidal activity of disinfectants against VSV. The effectiveness of a disinfectant should be tested against the bacteria or virus for which activity is claimed.

Klein and DeForest (Fed. Proc., p. 319, 1965) reported that ethyl alcohol and isopropanol were rapidly viricidal for lipophilic virus. In the present study, 40 to 90% ethyl alcohol and 70 to 90% iso-
alkaline chemicals, NaOH, KOH, Na₂CO₃, or Na₃SiO₃, was effective against VSV. However, in the United States, when a vesicular condition is found, it must be assumed to be FMD and chemicals used must be recognized as effective against FMD virus until the agent is proved to be VSV.

Whereas acids have also been used to control VSV, it was determined that acetic acid was of only marginal value, since variable results were obtained with a 5% concentration and lower concentrations were not viricidal. The results obtained with mineral acids depended on the particular acid, possibly owing to the different degrees of ionization (pK). The pK values of the mineral acids were hydrochloric acid, 0.784; sulfuric acid, 0.510; and acetic acid, 0.004.

The most consistent viricides for VSV were the phenolic types when a sufficient concentration was used, but the effective concentration was higher than the manufacturer's recommendations in some cases.

Organic iodine (U) and sodium hypochlorite were both active viricides. The virus was inactivated in 10 min even with the presence of the chorioallantoic membrane and allantoic fluid in the virus mixture. However, halogens are more

| Table 2. Ineffective disinfectants and concentrations tested against vesicular stomatitis virus |
|---|---|
| Disinfectant | Range of concent tested |
| Formalin | 3.0–10.0% |
| G₆ | 0.33–5.0% |
| Ethyl alcohol | 45.0–90.0% |
| Isopropanol | 70.0–90.0% |
| NaOH | 3.0–10.0% |
| KOH | 3.0–10.0% |
| Acetic acid | 1.0–5.0% |
| H₂SO₄ | 0.1–0.4% |
| Na₂CO₃ | 4.0–10.0% |
| Na₂SiO₃ | 5.0% |

a See Table 1.

| Table 3. Effective disinfectants and the concentrations tested against vesicular stomatitis virus |
|---|---|---|
| Disinfectant | Minimum effective concn | Range of concent tested |
| | % | % |
| A | 1.3 | 0.5–2.0 |
| B | 1.3 | 0.5–2.0 |
| C | 1.0 | 0.5–2.0 |
| D | 2.0 | 1.0–2.0 |
| E | 0.5 | 0.1–1.0 |
| J | 4.0 | 0.5–4.0 |
| L | 2.0 | 0.25–2.0 |
| N | 0.166 | 0.1–0.2 |
| Phenol | 2.5 | 1.0–5.0 |
| T | 1.6 | 0.75–1.6 |
| U | 4.0 | 1.0–4.0 |
| HCl | 0.4 | 0.1–0.4 |
| S | 1.0 | 0.5–1.0 |
| NaOCl | 0.645 | 0.22–1.1 |

a See Table 1.
b Results variable depending on lot tested.

propanol were not viricidal at a rate rapid enough to be useful against VSV.

Alkaline chemicals have been employed as viricides in vesicular disease outbreaks in the United States because of their application for the inactivation of foot-and-mouth disease (FMD) virus.

FMD virus was much more susceptible to change in pH as a means of virus destruction than VSV (4). VSV was resistant to destruction by alkaline chemicals in the present study. None of the alkaline chemicals, NaOH, KOH, Na₂CO₃, or Na₃SiO₃, was effective against VSV. However, in the United States, when a vesicular condition is found, it must be assumed to be FMD and chemicals used must be recognized as effective
susceptible to inactivation by organic material than other disinfectants.

The rate of inactivation was determined for three different types of disinfectants. Disinfectant L (2%) was rapidly viricidal by an apparent first-order reaction, but, when it was diluted to 0.5%, a diphasic inactivation curve was evident. The diphasic curve was also evident for acetic acid and NaOH, even though the pH of the NaOH remained stable at pH 12.2 over the time of exposure.

It appeared that the survivor curve was diphasic when the concentration of disinfectant was below that which was rapidly viricidal. Disinfectants at viricidal concentrations produced logarithmic virus survival curves or curves with a slight change in slope.

The survival of virulent virus particles may have been due to the size of the aggregate and degree of aggregation. When a sufficiently high concentration of phenolic and halogen type of disinfectants was present, the additional time required to inactivate the virus was minimal.

Based on laboratory evidence, substituted phenolics, halogens, or cresylic acids are recommended for use when vesicular stomatitis virus is to be destroyed on an infected premise. These chemicals are in the proper concentration under clean conditions and can be used with greater personal safety than acids and bases.

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