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Quantitative Studies on Fabrics as Disseminators of Viruses

II. Persistence of Poliomyelitis Virus on Cotton and Wool Fabrics

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Abstract

Dixon, Glen J. (Southern Research Institute, Birmingham, Ala.), Robert W. Sidwell, and Ethel McNeil. Quantitative studies on fabrics as disseminators of viruses. II. Persistence of poliomyelitis virus on cotton and wool fabrics. Appl. Microbiol. **14**:183–188. 1966.—The length of time that poliovirus could be recovered from wool gabardine and blanket, and from cotton sheeting, terry cloth, and knit jersey fabrics was determined under conditions of controlled temperature and humidity (25 C in 35 and 78% relative humidities). Three types of exposure of the fabrics to viruses were used: direct contact, aerosol, and virus-containing household dust having a high content of textile fibers. When held in 35% relative humidity, virus persisted for 20 weeks on wool fabrics, but only 1 to 4 weeks on cotton fabrics. At this relative humidity, virus titers on wool fabrics decreased rapidly to low but detectable levels which persisted for long periods of time, whereas in 78% relative humidity the decrease in virus titer was less rapid, but the period of viral persistence was shorter. Generally, virus titers on cotton fabrics held in both relative humidities decreased exponentially to an undetectable level. The method of exposure to virus had a definite effect on the duration of viral persistence on a given fabric. Virus contained in household dust was least stable.

The possible public health hazard associated with clothing contaminated with infectious disease agents has long been recognized (5). We have reported on the persistence of vaccinia virus on cotton and woolen fabrics under conditions of controlled temperature and humidity (11). Poliovirus and other enteroviruses present serious public health problems, and possibly may be disseminated by contaminated clothing. Poliovirus is one of the more stable viral agents. It will withstand repeated freezing and thawing, extended storage at −70 C (6), and, if suspended in 50% glycerol, it will survive for years at 4 C (4). It will survive for hours in human feces at "high temperature," i.e., summer heat (12). The virus reportedly is not completely inactivated by a commercial disinfectant (polyethoxy-ethanol-iodine), at a 1:200 dilution (recommended working concentration), when organic material is present (13). The virus is quite stable over a wide range of *p*H (3.8 to 8.5) for weeks (8).

Since the major source of poliovirus in the human environment is feces of infected individuals (cases or carriers), the persistence of the virus on fabrics commonly used in clothing and bedding is of major importance in considering possible virus dissemination by fomites.

The present report describes the persistence of poliovirus on wool and cotton fabrics exposed to the agent by three different methods after storage of the virus-contaminated fabrics in high and low relative humidities.

Materials and Methods

Fabrics tested. The fabrics used in these studies were wool blanket, wool gabardine, cotton sheeting, cotton knit jersey, and cotton terry cloth. All fabrics had been bleached, were undyed, and were not impregnated with antimicrobial or moth-proofing substances. More complete descriptions of these fabrics are reported elsewhere (11).

Virus. Poliovirus type 2 strain MEF-1 was used in these studies. This virus was obtained as a cell culture suspension from Parke, Davis & Co., Detroit, Mich., and was passed once through HEp-2 cells (7) to prepare a virus stock.

Experimental design. The persistence of poliovirus on fabrics was determined with three methods of exposure of fabric to virus and two relative humidities as variables. For each experiment, 80 sterile swatches (5 cm in diameter) of each fabric were exposed to a constant amount of poliovirus suspended in Eagle's basal medium (BME) (2) supplemented with 5.0% agamma calf serum and 0.5% chick embryo extract and adjusted to *p*H 7.2. The types of exposure of the fabrics to the virus were: direct contact (pipetting the virus directly on the fabric), aerosol, or virus-containing household dust made up predominantly of textile fibers. The virus-containing dust was prepared by sterilizing ordinary household dust, wetting it with a specific quantity of poliovirus suspension, and lyophilizing the resulting mixture (11). After exposure to virus, five swatches of each type of fabric were randomly selected, separated by sterile, glass-fiber screens, placed in an elevated-lid petri dish (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.), and incubated at 25 C in 35 or in 78% relative humidity. Virus titers of five swatches were determined at the following time intervals: immediately after exposure to virus (time zero), 2 hr, 7 and 14 days. If virus was undetectable at 7 and 14 days after virus exposure by direct contact, additional fabric swatches were exposed to virus by direct contact, and the virus titer was determined daily until virus was no longer detectable. If virus persisted through 14 days, fabrics were tested for virus titer at biweekly intervals until two negative tests were obtained.

To demonstrate the presence and to determine the titer of virus on each fabric, each swatch was macerated in 25 ml of BME in a Servall Omnimix homogenizer (Ivan Sorvall, Inc., Norwalk, Conn.) run at maximal speed (manufacturer states maximal speed to be 16,000 rev/min) for 30 sec. The eluate was removed and centrifuged lightly to remove suspended fibers, and the supernatant fluid was frozen in sealed ampoules at ca. −70 C. This material was later thawed, diluted 10-fold from 10^0 through 10^{-9}, and each dilution was assayed for virus in HEp-2 cells grown in vinyl plastic panels (10). A cell culture 50% infectious dose ($CCID_{50}$/ml), based on the cytopathogenic effect (CPE) observed, was calculated for each eluate by use of the method of Reed and Muench (9).

A more detailed description of the above procedures was reported previously (11).

RESULTS

The titer of virus from each fabric swatch was determined for the time intervals tested, and the mean titer for each group of five swatches was calculated. These mean titers are given in Fig. 1–5. Little variance from each mean was noted; 95% confidence limits calculated at each time interval were generally less than $\pm 0.5 \log_{10}$ from each mean.

On fabrics exposed to virus by direct contact or aerosol, the initial virus titers were high and relatively constant (ca. 10^9 to 10^{10} $CCID_{50}$/ml), but for those swatches exposed to virus-containing dust the initial titers were much lower (ca. 10^5 to 10^6 $CCID_{50}$/ml), although the amount of virus present on the different types of fabric

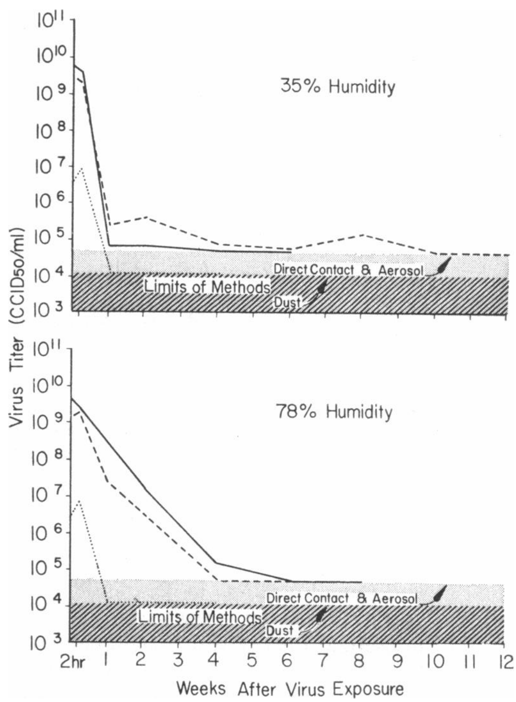

FIG. 1. *Persistence of poliovirus on wool gabardine material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.*

swatches was in close agreement. After initial exposure to virus, the persistence of the agent, with time, varied according to the type of fabric, method of exposure to virus, and relative humidity in which the swatches were held. The virus was recovered for longer periods of time from wool blanket material than from other types of fabrics. On wool blanket material exposed by direct contact, the virus was recovered through 20 weeks after exposure when held in 35% relative humidity. The cotton fabrics yielded virus for a maximum of only 1 to 4 weeks after exposure to virus. When swatches were exposed to virus by aerosolization and held in the low relative humidity, the virus persisted for longer periods of time than when held in the high relative humidity. Initially, the virus titers declined rapidly on the swatches held in 35% relative humidity, but after this initial rapid decline the titers remained relatively constant, and the virus generally persisted longer than on fabrics exposed to virus aerosol and held in 78% relative humidity. In contrast, when placed on wool gabardine and cotton fabrics by direct contact, virus was recovered for longer periods of

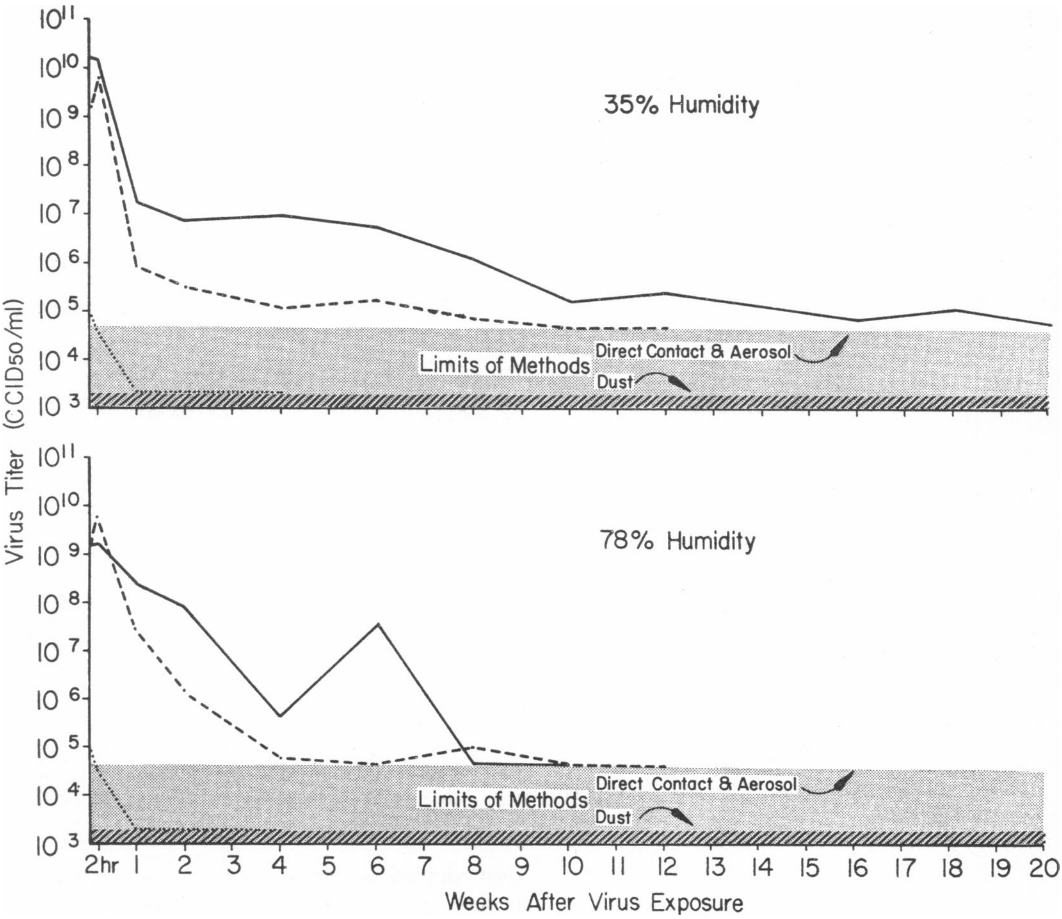

FIG. 2. *Persistence of poliovirus on wool blanket material held at 25 C in two humidities. Data expressed a mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-con taining dust.*

time in the high relative humidity than in the low relative humidity. Virus was undetectable by 1 week after exposure in all experiments in which swatches were exposed to dust containing poliovirus.

Because of the rapid decline of virus titers on cotton fabrics (sheeting and knit jersey held in 35% relative humidity, terry cloth held in 78% relative humidity) and on wool gabardine material (35% relative humidity), all exposed to virus by direct contact, the experiments were repeated with swatches tested daily to determine more specifically the rate of virus inactivation (Fig. 6). The detectable virus on the terry cloth fabric decreased to undetectable levels by 1 week after exposure, whereas on sheeting and knit jersey no virus could be demonstrated by day 4. The wool gabardine material yielded low titers of virus through day 10, the final day of the experiment.

DISCUSSION

To obtain a broader understanding of the dissemination of viruses by fomites, a knowledge of three basic elements is needed: (i) factors influencing the contamination of the materials, (ii) factors influencing survival and infectivity, and (iii) conditions under which the viruses are released (5). The primary goal of the present series of investigations is to gain an understanding of the first two of these elements, by studying the effects on the persistence of virus of three methods of virus exposure, type of fabric and weave, and relative humidity.

These studies indicate that the method by which poliovirus is placed on fabrics has a significant effect on the persistence of the virus. Exposure of fabrics to virus by aerosolization and direct contact resulted in prolonged recovery of the virus, but fabrics exposed to virus-containing

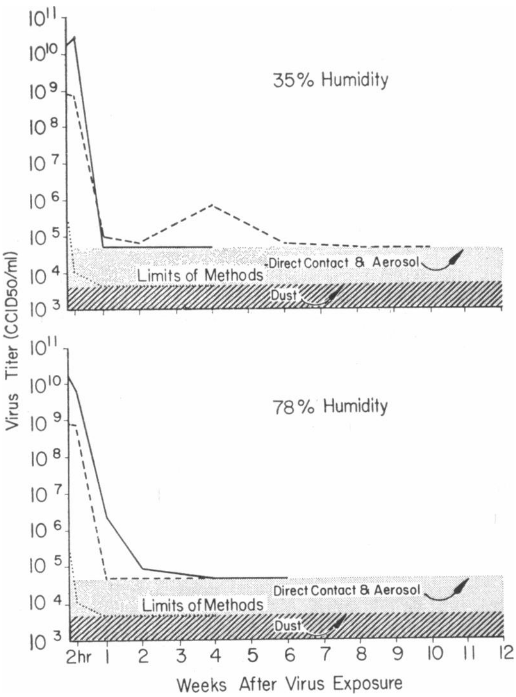

FIG. 3. *Persistence of poliovirus on cotton sheeting material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.*

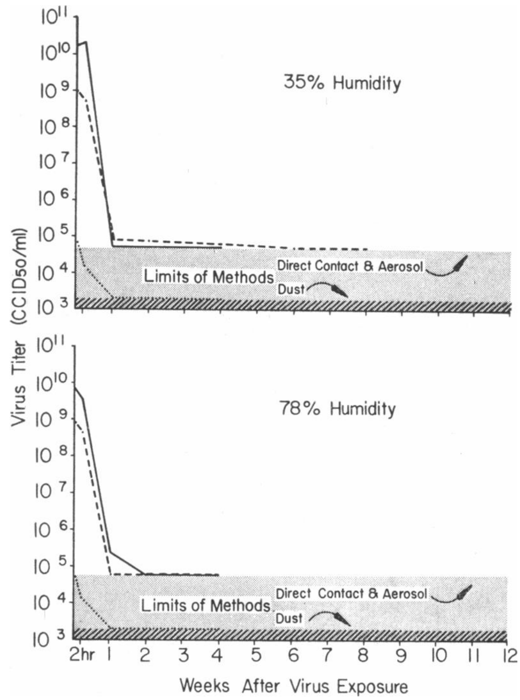

FIG. 5. *Persistence of poliovirus on cotton terry cloth material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.*

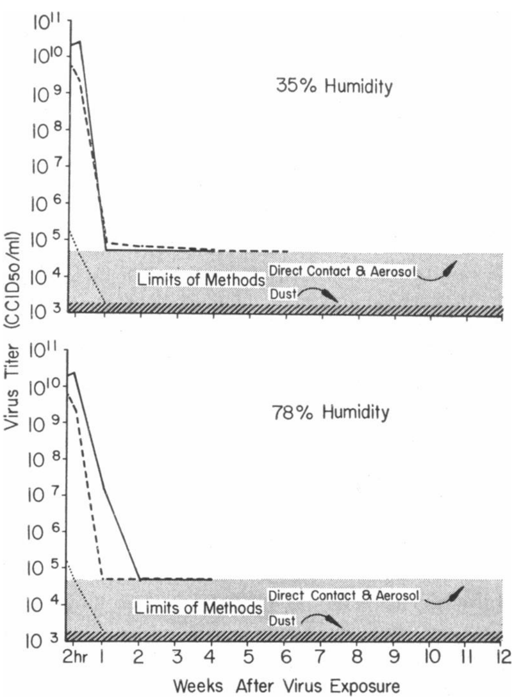

FIG. 4

dust yielded no virus within 1 week after exposure, a result which is in direct contrast to observations made with vaccinia virus (11). Results of this nature might be expected, however, on the basis of reports by others that poliovirus is highly unstable when lyophilized (4).

The type of fabric and weave apparently influenced the recovery of the agent, and the method of virus exposure had a profound effect on the persistence of the virus. However, the decrease in virus titer with time on any given fabric was consistent regardless of which method of virus exposure was used. Poliovirus persisted on wool materials for the longest periods of time, which parallels the results obtained with the vaccinia virus studies (11). An explanation of these observations is difficult. Certain types of fabrics may provide a protective environment for the virus. In these studies, it was observed that wool blanket material, when eluted with BME, caused the eluate to be acidic. Consequently, a more

FIG. 4. *Persistence of poliovirus on cotton knit material held at 25 C in two humidities. Data expressed as mean virus titers. Method of exposure to virus: solid line, direct contact; dashed line, aerosol; dotted line, virus-containing dust.*

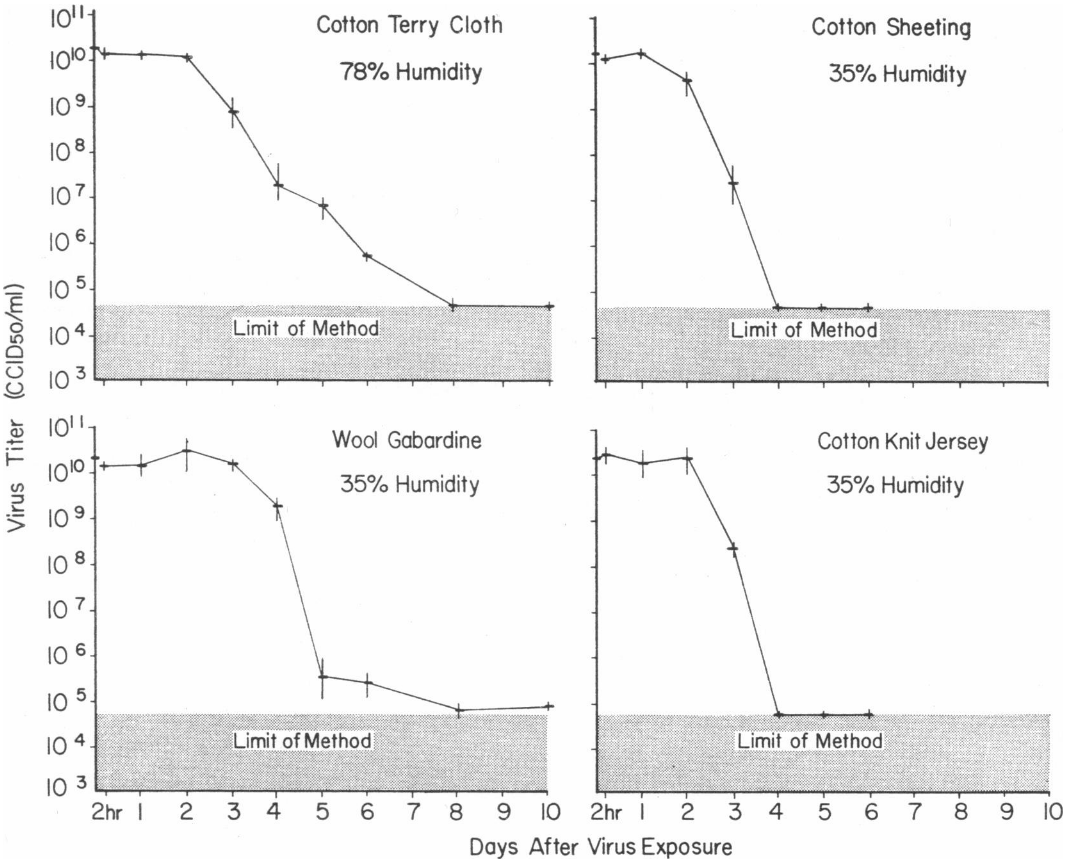

FIG. 6. *Persistence of poliovirus pipetted on cotton terry cloth, cotton sheeting, wool gabardine, and cotton knit jersey materials held at 25 C in 35 or 78% humidities. Data expressed as mean virus titers ±95% confidence limits at each time tested.*

basic medium (ca. *p*H 9) was employed as eluent for this material, which yielded an eluate with a *p*H of about 7. As was previously reported (11), the maceration-elution process may have been more effective for the wool fabrics, since the end result of this process was a fine lintlike mass for the wool fabrics. Although lintlike material was produced from the cotton fabrics in the homogenizer, the fabric mass was more compact. The gradual decline of virus titer with time, however, would indicate an effective removal of the virus from the fabrics.

Possibly the most significant of these results were the observations that relative humidity had a pronounced effect upon viral persistence. Hemmes, Winkler, and Kool (3) reported that aerosolized poliovirus was inactivated rapidly in low relative humidity and slowly in high relative humidity, and correlated the high morbidity of human poliomyelitis infections with periods of high relative humidity. In the present study, virus declined rapidly in titer when held in low relative humidity, but became constant at a low level and persisted for a longer period of time than when held in 78% relative humidity. This type of result was more apparent with cotton sheeting exposed to aerosolized virus and with wool blanket and wool gabardine materials. These studies tend to strengthen the hypothesis of Hemmes et al. (3) and DeJong and Winkler (1) that relative humidity is an important factor in the seasonal variation of poliomyelitis.

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LITERATURE CITED

1. DEJONG, J. G., AND K. C. WINKLER. 1964. Survival of measles virus in air. Nature **201**:1054–1055.

2. EAGLE, H. 1955. The minimum vitamin requirements of the L and HeLa cells in tissue culture, the production of specific vitamin deficiencies and their cure. J. Exptl. Med. **102**:595–606.
3. HEMMES, J. H., K. C. WINKLER, AND S. M. KOOL. 1960. Virus survival as a seasonal factor in influenza and poliomyelitis. Nature **188**:430–431.
4. HOWE, H. A., AND J. L. WILSON. 1959. Poliomyelitis, p. 447. *In* T. M. Rivers and F. L. Horsfall, Jr. [ed.], Viral and rickettsial infections of man. J. B. Lippincott Co., Philadelphia.
5. MCNEIL, E. 1964. Dissemination of microorganisms by fabrics and leather. Develop. Ind. Microbiol. **5**:30–35.
6. MELNICK, J. L. 1946. Storage of mouse-adapted strains of poliomyelitis virus and Japanese B encephalitis virus at subfreezing temperatures. J. Infect. Diseases **79**:27–32.
7. MOORE, A. E., L. SABACHEWSKY, AND H. W. TOOLAN. 1955. Cultural characteristics of four permanent lines of human cancer cells. Cancer Res. **15**:598–602.
8. POLLARD, M., AND J. CONNOLLY. 1949. pH stability of MEF poliomyelitis. Texas Rept. Biol. Med. **7**:92–95.
9. REED, L. J., AND A. MUENCH. 1938. A simple method of estimating fifty percent end points. Am. J. Hyg. **27**:493–498.
10. RIGHTSEL, W. A., P. SCHULTZ, D. MUETHING, AND I. W. MCLEAN, JR. 1956. Use of vinyl plastic containers in tissue culture for virus assays. J. Immunol. **76**:464.
11. SIDWELL, R. W., G. J. DIXON, AND E. MCNEIL. 1966. Quantitative studies on fabrics as disseminators of viruses. I. Persistence of vaccinia virus on cotton and wool fabrics. Appl. Microbiol. **14**:55–59.
12. TRASK, J. D., J. R. PAUL, AND A. J. VIGNEC. 1940. I. Poliomyelitic virus in human stools. J. Exptl. Med. **71**:751–763.
13. WALLIS, C., A. M. BEHBEHANI, L. H. LEE, AND M. BIANCHI. 1963. The ineffectiveness of organic iodide (Wescodyne) as a viral disinfectant. Am. J. Hyg. **78**:325–329.