Role of Virucides in Controlling Virus Dissemination by Fabrics[1]

ROBERT W. SIDWELL and GLEN J. DIXON, Virus and Cell Biology Divisions, Southern Research Institute, Birmingham, Alabama 35205

Abstract

Vaccinia virus, a lipophilic agent containing deoxyribonucleic acid, and poliovirus, a hydrophilic ribonucleic acid virus, persisted on wool and cotton fabrics for varying periods up to 20 weeks, which was of sufficient duration to be of epidemiological significance. The length of persistence of each virus varied with the type of fabric, humidity and method of exposure to the virus. A group of quaternary ammonium salts and bromosalicylanilides were evaluated quantitatively for virucidal activity against these viruses in a cell culture system. None of the compounds was active against poliovirus, but three of the quaternary ammonium compounds significantly inactivated vaccinia virus. Impregnation of wool and cotton fabrics with one of these compounds resulted in a marked decrease in vaccinia virus persistence. Both polio and vaccinia viruses persisted for less than five days on a cotton fabric finished with a modified triazone resin to impart a wash-and-wear property. Cotton fabric contaminated with vaccinia or with poliovirus was laundered with an anionic detergent and a nonionic detergent. This laundering reduced but did not eliminate the virus. Sterile fabric was contaminated with virus when laundered with the virus-containing fabrics. Drying the fabrics for 20 hr after laundering reduced the virus titers to below detectable limits.

Introduction

It is a relatively easy task in these days of rapid transportation and high population densities to transmit disease organisms throughout the world unknowingly, particularly when certain pathogens have the ability to induce inapparent or latent infections in man. In these instances, persons clinically free of disease symptoms can be inadvertent carriers of the disease agent. Clothing worn by these carriers could be implicated as one means of transmitting the infectious agent from these persons. Thus, the potential problem of microorganism dissemination by fabrics becomes increasingly important.

Suspicions concerning fabrics as potential fomites (objects capable of transmitting contagious disease agents) have been strengthened by numerous reports in the past few decades describing studies with various species of bacteria, fungi and protozoa on textile articles (1). Until very recently, however, little has been known concerning the possible role fabrics may play in dissemination of viruses. This report describes studies we have carried out to determine more fully this fomite role in regard to viruses. Also discussed are experiments we have initiated in attempting to reduce this potential fomite hazard through impregnation of fabrics with virucidal chemicals and to determine to what extent selected viruses survive home-type laundering and are transferred from contaminated to noncontaminated fabrics during laundering.

There are certain basic factors which must be considered in order to develop means for preventing or controlling the spread of disease agents by inanimate objects such as textile articles. These factors include the initial concentration of the disease agent, the method of contamination, the suspending medium of the disease agent, the rate of inactivation and the rate of release of the disease agent. Hence, for fabrics to be successful fomites, the objects under study must be contaminated in some manner, then become carriers of the contaminant. The contaminant must be able to survive physical and chemical stresses to which it may be normally exposed. This ability to survive can be greatly influenced by the type of vehicle in which the virus is contained. Finally, the object must be capable of releasing the contaminant into the environment. Each of these factors was considered in the present studies.

Experimental Procedures

Viruses

Two viruses were used in these studies: vaccinia virus (Strain Lederle Chorioallantoic) and poliovirus Type 2 (Strain MEF-1). Both viruses were obtained in cell culture suspension from Parke, Davis and Company, Detroit, Michigan, and were grown in HEp-2 cells (2) to prepare a virus stock.

Fabrics

Cotton and wool fabrics were used in these studies. These fabrics were described in detail previously (3). A wash-and-wear fabric used in a portion of these studies had a 100% plied yarn cord and was finished with a starch filler (Laurel), softener (Emersoft 7701), a modified triazone resin (Perma Fresh 197), a magnesium chloride catalyst, an adhesive (Rhoplex HA-24), and a whitener (Blue RP and Leucophor AC). After the above finishing, the fabric was sanforized. All fabrics were white and had not been treated with moth-proofing or antimicrobial agents. Prior to use in these studies, each fabric was tested for inherent antimicrobial activity with *Staphylococcus aureus* and *Escherichia coli* in the agar plate test described in the AATCC Technical Manual (4). No activity was demonstrated.

Determination of Viral Persistence on Fabrics

Figure 1 summarizes the methodology used for this portion of the study. Two-inch diameter swatches of each fabric, which had been sterilized with ethylene oxide gas, were exposed to approximately 10^{10} infectious units per milliliter of virus contained in cell culture media (Eagle's basal medium supplemented with 5.0% agamma calf serum and 0.5% chick embryo extract). Three methods were used for this exposure: direct contact, exposure to a virus aerosol, and exposure to virus-containing household dust. At varying times after exposure, during which the virus-contaminated fabrics were stored at 25 C in 35% or

[1] Presented at the AOCS Meeting, New York, October, 1968.

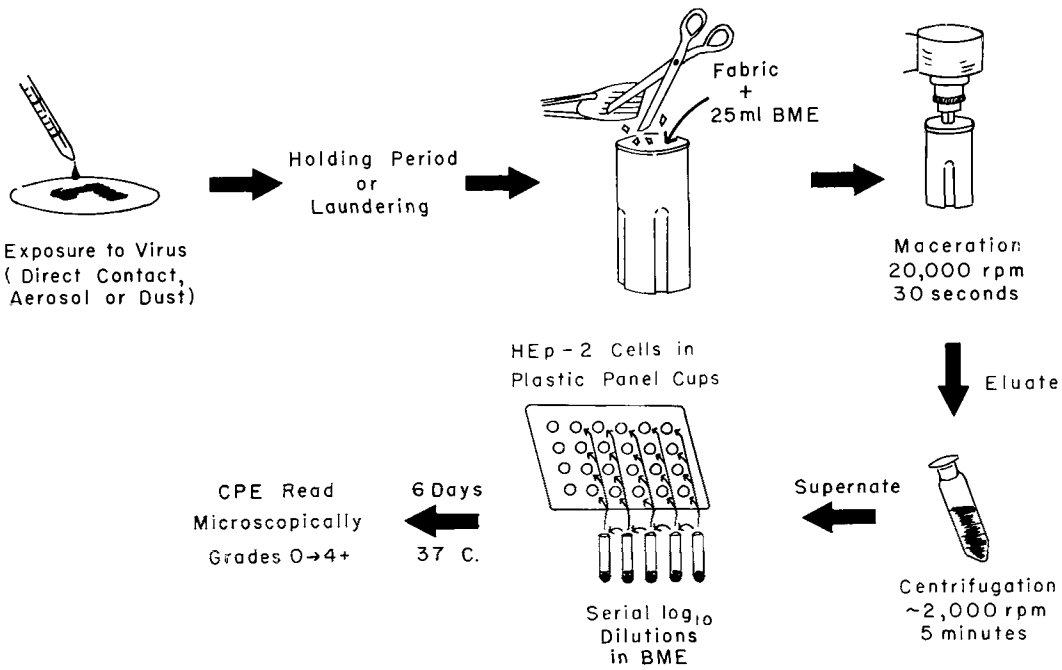

FIG. 1. Summary of the methodology used for determining the viral content of fabrics.

78% relative humidity, the fabrics were tested for the presence and quantity of viable virus. This was done by macerating the swatches in cell culture medium using an Omnimix homogenizer run at 16,000 rpm for 30 sec. This effectively reduced each fabric to shredded fibers and the majority of the viable virus was released into the medium. The virus titers were determined by testing varying dilutions of the centrifuged eluate in a culture of susceptible animal cells (HEp-2). Viable virus causes extensive cytopathic effects (CPE) in these cells, which were read microscopically in six days and the degree of CPE graded on a scale from 0 to 4. The details of humidity maintenance, method of virus exposure, and recovery of the virus have been described previously (3).

Determination of Virucidal Effects of Test Chemicals

Each test chemical was dissolved in cell culture medium or sterile physiological saline and diluted through a series of one half $\log_{10}$ dilutions. Each solution was then mixed with equal volumes of virus suspension and incubated for 1 hr at 37 C. After incubation the mixtures were diluted 1:100 in HEp-2 cell suspensions and incubated at 37 C for six days. Virucidal activity was determined by reduction in viral CPE as compared with the virus controls, which had not been exposed to a test chemical. The concentrations of each test product were selected to range from cytotoxic to nontoxic levels. The virucidal activity of each was evaluated statistically by use of a virus rating (VR), in which toxicity, degree of CPE inhibition, and minimum active dilution of the test compound were considered. The method of Ehrlich et al. (5) was used with minor modifications as described in an earlier report (6). A VR of 1.0 or greater was interpreted to indicate definite activity, whereas a rating of 0.5 to 0.9 indicated questionable activity, and any rating of less than 0.5 was considered insignificant and interpreted as no apparent virucidal activity.

Impregnation of Fabrics With a Virucidal Chemical

Wool blanket, wool gabardine and cotton sheeting materials were conditioned for 24 hr at 22 C in 65% relative humidity, weighed and added to a 1:10 dilution of the test chemical. They were then processed twice through a Butterworth padder using a pressure of 11–12 lb/in.^{2}. Each fabric was dried, conditioned as described above for 24 hr, and reweighed. The before and after weights indicated that the chemical add-on due to this processing was 3.3% for wool blanket, 7.6% for wool gabardine, and 4.4% for cotton sheeting.

Laundering of Virus-contaminated Fabrics

Strips of fabric cut to a size of 12 × 36 in., as well as 2 in. diameter swatches of the fabric which were attached to the strips, were exposed to the viruses by direct contact, allowed to dry for 16 hr in 35% relative humidity, and laundered in an automatic hometype washing machine (Lady Kenmore, Sears, Roebuck and Co.) using wash, rinse and spin-dry cycles. The concentration of detergent was approximately 0.2%, using a washer load of 11.4 gal (44 liters). A low wash temperature of 21.1–26.7 C was used. The water hardness was approximately 30 ppm of $CaCl_2$ and $MgCl_2$. When the fabrics were laundered, equal numbers of sterile and virus-contaminated strips were added in sufficient number to the wash to provide, by weight, a standard wash load. The wash cycle ran for 14 min, followed by a 2 min spray rinse, a 4 min deep rinse, another 2 min spray rinse, and a 4 min spin-dry cycle. Each set of virus-contaminated fabrics was laundered separately in individual experiments.

Results and Discussion

As we have reported previously (3,7), the persistence of vaccinia virus on the fabrics tested varied considerably, depending on the fabric, humidity and method of exposure. The virus persisted for relatively long periods (up to 14 weeks) on wool and cotton fabrics, particularly if stored in low humidity. The agents survived only one day or less on the wash-and wear fabric. Somewhat similar results were observed with the poliovirus, with high concentrations of the virus remaining over five months on wool

TABLE I
Summary of Experiments on the Virucidal[a] Activity of Selected Test Compounds

Compound	Activity vs. poliovirus		Activity vs. vaccinia virus	
	Max. VR[b]	Concl. effect	Max. VR[b]	Concl. effect
Benzalkonium chloride[c]	0.4	—	0.9	±
Diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, monohydrate[d]	0	—	0.2	—
Methyldodecylbenzyl trimethyl ammonium chloride (40%) + methyl dodecylxylylene bis(trimethyl ammonium chloride) (10%) + H_2O (50%)[d]	0	—	0.4	—
n-Alkyl (C_{14}, C_{12}, C_{16}) dimethyl benzyl ammonium chloride (50%) + EtOH and H_2O (50%)[d]	0	—	1.5	+
Diisobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate[d]	0	—	1.6	+
3,5-Dibromosalicylanilide (12–24%) + 3,4',5-tribromosalicylanilide (76–88%)[e]	0	—	0.1	—
3,4',5-Tribromosalicylanilide (98–100%)[e]	0	—	0 3	—
n-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18})-dimethyl benzyl ammonium chlorides (25%) + *n*-Alkyl (50% C_{12}, 30% C_{14}, 17% C_{16}, 3% C_{18})-dimethyl ethylbenzyl ammonium chlorides (25%)[f]	0	—	1.3	+
Neomycin sulfate[g]	0	—	0	—

[a] Compounds mixed with virus, incubated 1 hr at 37 C before addition to cell culture.
[b] VR, virus rating; a measure of CPE inhibition by a modification of the method of Ehrlich et al. (5); >1.0 = +, or possibly activity; 0.5–0.9 = ±, or questionable activity; <0.5 = —, or no apparent activity.
[c] CIBA Chemical and Dye Co., Fairhaven, N.J.
[d] Rohm and Haas Co., Philadelphia, Pa.
[e] Dow Chemical Co., Midland, Mich.
[f] Onyx Chemical Co., Jersey City, N.J.
[g] Philadelphia Laboratories, Inc., Philadelphia, Pa.

blanket material, but having a short persistence of only three to five days on cotton fabrics. On the wash-and-wear material, the virus usually persisted less than one day.

These results support the premise that fabrics may play an important role in the dissemination of viruses. The efficacy of fabric impregnation was studied as one method to reduce this possible hazard of virus transmission. The use of this impregnation method was prompted by the results just seen of the reduced viral persistence on cotton fabric which had been impregnated with various chemicals to impart a wash-and-wear finish. Such fabric impregnation techniques

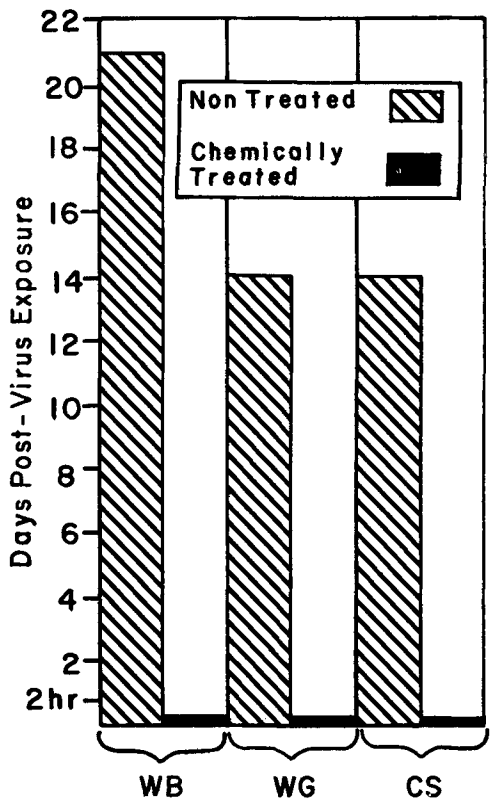

FIG. 2. Persistence of vaccinia virus on three fabrics impregnated with *n*-alkyl (C_{14},C_{12},C_{16}) dimethyl benzyl ammonium chloride and held at 25 C in 35% relative humidity. WB, wool blanketing; WG, wool gabardine; CS, cotton sheeting.

are not unique as a means of reducing microbial contamination. The method has been used to control the dissemination of infectious agents, to reduce bacteria-induced odors, to prolong the life of fabrics by preventing staining and degradation by microorganisms, and for imparting a contaminant-free environment to surgical dressings. The use of such a process in approaching the viral problem has not been reported by other investigators.

A series of experiments was carried out to ascertain first, the virucidal activity of a variety of chemicals, to determine the best virucide to use for fabric impregnation studies. These chemicals included several quaternary ammonium compounds, two bromosalicylanilides and an antibiotic. Results of this study are summarized in Table I. Of the compounds tested, only the quaternary ammonium compounds had activity against vaccinia virus. Poliovirus was not affected by any of these materials. These results are similar to those reported by others (8–15) using other virucidal test procedures.

One of the quaternary ammonium compounds, *n*-alkyl(C_{14},C_{12},C_{16})dimethyl benzyl ammonium chloride, was used to impregnate wool blanketing, wool gabardine and cotton sheeting materials. Following impregnation, each treated fabric was cut into swatches, sterilized with gaseous ethylene oxide, and after an appropriate holding period to ensure that residual ethylene oxide was no longer present on the fabric, each swatch was exposed to vaccinia virus by direct contact. The titer of virus on five swatches was determined at time intervals starting from time 0. Virus titers of control fabrics (untreated with chemical) were determined at the same time as the treated fabrics. Sterile swatches of each treated fabric were tested to determine their toxicity in cell culture. No virus could be recovered at any time from the impregnated fabrics (Fig. 2), although the duration of viral persistence on similar nonimpregnated fabrics varied from 5 to 21 days. It was concluded that the pretreatment of the fabrics with the quaternary ammonium compound resulted in an immediate reduction of more than 4 $\log_{10}$ of infectious virus. Only slight cytotoxicity was demonstrated by the treated fabrics.

These studies suggest that treatment of fabrics to render them self-sanitizing against certain viruses

TABLE II
Effect of Laundering[a] in an Anionic or a Nonionic Detergent[b] on Polio and Vaccinia Virus Titers on Cotton Sheeting[c]

Detergent	Virus	Virus titers[d]					
		16 hr virus control[e]	Held 16 hr laundered[f]	Laundered sterile control[g]	Rinse water[h]	36 hr virus control[i]	Held 16 hr, laundered, dried 20 hr[j]
Anionic	Polio	$10^{5.6}$	$10^{3.4}$	$10^{1.2}$	$<10^{0.9}$	$10^{0.5}$	$<10^{0.4}$
Anionic	Vaccinia	$10^{4.1}$	$<10^{0.4}$	$10^{0.4}$	$<10^{0.9}$	$10^{2.3}$	$<10^{0.4}$
Nonionic	Polio	$10^{5.8}$	$10^{3.6}$	$<10^{0.9}$	$10^{0.9}$	$10^{1.0}$	$10^{0.5}$
Nonionic	Vaccinia	$10^{4.9}$	$10^{2.6}$	$<10^{0.9}$	$10^{1.5}$	$10^{3.7}$	$<10^{0.4}$

[a] Carried out in an automatic washer using 14 min wash, 2 min spray-rinse, 4 min deep rinse, 2 min spray-rinse, and 4 min spin-dry cycles, at a temperature of 21.1–26.7 C.
[b] Total of 0.2% concentration of the detergents described in the text.
[c] Fabric was exposed to the viruses by direct contact (pipette).
[d] Each virus titer is expressed as the mean titer, in $CCID_{50}$/ml, of five samples.
[e] Exposed to virus, held 16 hr in 35% humidity, but unlaundered.
[f] Exposed to virus, held 16 hr in 35% humidity, then laundered and tested for virus.
[g] Sterile fabric laundered with the virus-contaminated fabric described in Footnote [f] and tested for virus.
[h] Samples taken near the end of the deep rinse cycle.
[i] Exposed to virus, held 36 hr in 35% humidity, but unlaundered.
[j] Exposed to virus, held 16 hr in 35% humidity, laundered, held 20 hr in 35% humidity, and tested for virus.

may be of practical use, particularly for materials to be used in areas suspected of being contaminated with those viruses. If effective chemicals can be found which have significant activity against a broad spectrum of viruses, such a process may have wide application.

Recently, we have begun a study to determine to what extent selected viruses survive home-type laundering and are transferred from contaminated to noncontaminated fabrics during the laundering processes. Polio and vaccinia viruses were used for this study, and one anionic and one nonionic detergent were investigated. Both detergents are typical of the detergents now on the domestic market. The anionic detergent, obtained from the Procter and Gamble Co., Cincinnati, Ohio, had the following major ingredients: Sodium linear alkylate sulfonate, nonionic surfactant (alkyl ethylene oxide condensate), sodium soap, sodium tripolyphosphate, sodium silicate and sodium sulfate. The nonionic detergent was obtained from the Lever Brothers Company, Inc., Edgewater, N.J. This product has, as its major ingredients, nonionic detergent (synthetic alcohol-ethylene oxide condensate), sodium tripolyphosphate, cellulosic, sodium silicate, sodium sulfate, perfume and optical dye.

Cotton sheeting material was exposed by direct contact to one of the viruses and held for 12 hr at 25 C in 35% humidity. The effect of laundering of this material using each type of detergent was then evaluated. In this evaluation, five swatches of unlaundered virus-contaminated fabric (virus control), laundered virus-contaminated fabric (test) and sterile material (sterile control) which had been laundered with the virus-contaminated fabric were tested to determine the amount of virus, if any, that remained on each. Ten milliliters of the rinse water were removed near the end of the rinse cycle and also assayed for content of viable virus. To determine the effects of drying on any virus which may have remained on the fabric, five swatches of virus control and of test fabric were held at 25 C in 35% relative humidity 20 hr after laundering and then assayed for viable virus.

The results of laundering the fabrics in the anionic detergent are summarized in Table II. The mean titer of poliovirus, as expressed in 50% cell culture infectious doses ($CCID_{50}$), was reduced only about 2 $\log_{10}$, but the titer of vaccinia virus was reduced approximately 4 $\log_{10}$ to below detectable limits. When the laundered poliovirus-contaminated swatches were held for an additional 20 hr at 25 C in 35% humidity, the titers declined to below detectable limits. Neither virus was recoverable from the rinse water, but over 1 $\log_{10}$ of poliovirus was detected on the sterile control fabric which had been laundered with the virus-contaminated materials.

Table II also summarizes the effects of laundering with the nonionic detergent on the two viruses. The virus titers were reduced over 2 $\log_{10}$, although a significant quantity of each virus remained on the fabric. Drying after laundering resulted in additional decreases in virus titers. No virus was detectable in the rinse water, but both viruses were recoverable from the sterile fabric laundered with the virus-contaminated materials.

It is probable that the virus titer reductions observed were primarily a result of the physical removal of a portion of the virus, rather than specific viral inactivation. This conclusion is based on the observation that virus was recovered from the sterile control fabric which had been laundered with the virus-contaminated fabric. The rinse water probably contained virus but in concentrations too low to be detectable in our assay system. These observations are particularly important because they demonstrate clearly the possible role fabrics may play in the dissemination of viruses.

Poliovirus and vaccinia virus were selected to represent a broad spectrum of viruses in these studies because of their widely differing properties. The poliovirus is hydrophilic, contains ribonucleic acid and is relatively small (approximately 30 mμ). The vaccinia virus is lipophilic, since it has a lipid-containing outer envelope. It contains deoxyribonucleic acid and is one of the larger of the animal viruses (approximately 250 mμ). Each virus apparently varies widely in susceptibility to various chemical disinfectants (13–16). From a public health standpoint, each represents important virus groups (picornaviruses and poxviruses, respectively).

Results of these experiments indicate that fabrics may play an important role in the dissemination of viruses. Fabric treatment with a virucidal chemical may be considered as a means for reducing this possible virus fomite role and, although laundering in the present studies also reduced the virus content of fabrics, the data suggest that viral transfer also occurs during the laundering process.

ACKNOWLEDGMENTS

Assistance with fabric impregnation by Harmon Hoffman. Much technical work was carried out by Miss Louise Westbrook and Miss Gussie Arnett. Project supported by USDA Contract No. 12-14-100-7189(63), 12-14-100-8317(63) and 12-14-100-9515(73).

REFERENCES

1. McNeil, E., Develop. Ind. Microbiol. *5*, 30 (1964).
2. Moore, A. E., L. Sabachewsky and H. W. Toolan. Cancer Res. *15*, 598 (1955).
3. Sidwell, R. W., G. J. Dixon and E. McNeil. Appl. Microbiol. *14*, 55 (1966).
4. Am. Assoc. Textile Chem. Colorists, "Technical Manual," Durham, N.C., 1963, B139.
5. Ehrlich, J., B. J. Sloan, F. A. Miller and H. E. Machamer, Ann, N.Y., Acad. Sci. *130*, 5 (1965).
6. Sidwell, R. W., G. J. Dixon and E. McNeil. Appl. Microbiol. *15*, 921 (1967).
7. Dixon, G. J., R. W. Sidwell and E. McNeil. Ibid. *14*, 183 (1966).
8. Armstrong, J. A., and E. J. Froelich. Ibid. *12*, 132 (1964).
9. Dunham, W. B., "Antiseptic Disinfectants, Fungicides and Sterilization." Edited by G. F. Reddish, Lea and Febiger, Philadelphia, 1967, p. 432.
10. Faber, H. K., and L. Dong, Am. J. Diseases Children *86*, 469 (1953).
11. Hammon, W. M., and W. C. Reeves. Proc. Soc. Exptl. Biol. Med. *60*, 84 (1945).
12. Klein, M., and A. DeForest. Proc. Chem. Specialties Mfrs. Assoc. *49*, 116 (1963).
13. Klein, M. S., S. S. Kalter and S. Mudd. J. Immunol. *51*, 389 (1945).
14. Toomey, J. A., and W. S. Takacs. Arch. Pediat. *62*, 337 (1945).
15. Lawrence, C. A., "Disinfection, Sterilization and Preservation," Edited by C. A. Lawrence and S. S. Block, Lea and Febiger, Philadelphia, 1968, p. 430.
16. Koski, T. A., and L. S. Stuart, "Disinfection, Sterilization and Preservation," Edited by C. A. Lawrence and S. S. Block, Lea and Febiger, Philadelphia, 1968, p. 194.

[Received March 4, 1969]