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Quantitative Studies on Fabrics as Disseminators of Viruses

III. Persistence of Vaccinia Virus on Fabrics Impregnated with a Virucidal Agent

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Eight compounds were tested in vitro for virucidal and antiviral activity against poliovirus and vaccinia virus. These compounds included five quaternary ammonium salts, two bromosalicylanilides, and neomycin sulfate, an antibiotic. None of the compounds was active against poliovirus, but virucidal activity was demonstrated against vaccinia virus with three of the quarternary ammonium compounds: *n*-alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride, di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate, and *n*-alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides plus *n*-alkyl (50% C12, 30% C14, 17% C16, 3% C18) dimethyl ethylbenzyl ammonium chlorides. Wool blanketing, wool gabardine, and cotton sheeting materials were impregnated with the first of the above virucidal compounds, and the persistence of vaccinia virus on these fabrics was compared with the persistence of the agent on nonimpregnated fabrics of the same type held at 25 C in 35 and 78% relative humidity. No virus could be recovered from the chemically treated fabrics at any time after virus exposure, whereas the virus persisted as long as 4 weeks on nonimpregnated materials. Viable vaccinia virus was also found to persist less than 1 day on a cotton fabric finished with a wash-and-wear modified triazone resin. Poliovirus persisted less than 5 days on this wash-and-wear fabric.

Previous reports (2, 12) have described the persistence of vaccinia virus and poliovirus on various fabrics under controlled conditions of temperature and humidity. These studies indicated that, owing to prolonged viral persistence on fabrics under certain conditions, clothing of various types could play a secondary role in the dissemination of viruses. Because of these findings, a search for effective virucidal or antiviral compounds for use in treating fabrics to render them refractory to viruses would seem to be a worthwhile effort. In the present report, the efficacy of fabric impregnation with antiviral or virucidal compounds in reducing the length of vaccinia virus persistence is evaluated. The persistence of poliovirus and vaccinia virus on a cotton fabric commercially treated with a wash-and-wear resin was also determined. The basic information and procedures derived from the previous studies were applied to this investigation. Included in this study are the results of experiments carried out to evaluate the antiviral and virucidal activity of eight compounds currently in commercial use for imparting antibacterial activity to fabrics.

MATERIALS AND METHODS

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 & Company, Detroit, Mich., and were grown in HEp-2 cells (11) to prepare a virus stock.

Fabrics. The following fabrics were used: wool blanketing (Fieldcrest Mills, Spray, N.C.), wool gabardine (J. P. Stevens Co., Inc., Milledgeville, Ga.), cotton sheeting (Pepperell Manufacturing Co., Abbeville, Ala.), and cotton wash-and-wear material (Russell Mills, Alexander City, Ala.). The latter fabric 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 to impart smoothness and to reduce shrinkage. No sanitizing or antimicrobial finishes were used in any of the above fabrics.

Compounds. Eight compounds were tested for antiviral and virucidal activity. These compounds were the following:

(1) Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, monohydrate.

(2) Methyldodecylbenzyl trimethyl ammonium chloride, 40%; methyl dodecylxylylene bis(trimethyl ammonium chloride), 10%; and water, 50%.

(3) *n*-Alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride, 50%; and ethyl alcohol and water, 50%.

(4) Di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate.

(5) 3,5-Dibromosalicylanilide, 12 to 24%; 3,4',5-tribromosalicylanilide, 76 to 88%; 4',5-dibromosalicylanilide, maximum 0.2%; 2',3,4',5-tetrabromosalicylanilide, maximum 0.5%.

(6) 3,4',5-Tribromosalicylanilide, 98 to 100%; 3,5-dibromosalicylanilide, <1.5%; 4',5-dibromosalicylanilide, <0.2%; and 2',3,4',5-tetrabromosalicylanilide, <0.3%.

(7) *n*-Alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides, 25%; *n*-alkyl (50% C12, 30% C14, 17% C16, 3% C18) dimethyl ethylbenzyl ammonium chlorides, 25%; and liquid inert ingredients, 50%.

(8) Neomycin sulfate.

Because of the complexity of the majority of these names, each compound will be referred to by the number preceding it. Compounds 1–4 were obtained from Rohm and Haas Co., Philadelphia, Pa; compounds 5 and 6 were received from the Dow Chemical Co., Midland, Mich., compound 7 was supplied by the Onyx Chemical Co., Division of Millmaster Onyx Corp., Jersey City, N.J., and compound 8 was received from Philadelphia Laboratories, Inc., Philadelphia, Pa. These compounds have been used to some extent for imparting bacteriostatic or fungistatic properties to textile fabrics, soaps, detergents, deodorants, and other cosmetic materials.

Procedure for determining antiviral effect. To determine the antiviral activity of the above compounds, 9.9 ml of an HEp-2 cell suspension containing ca.

TABLE 1. *Summary of studies on the virucidal[a] effect of selected compounds on vaccinia virus in HEp-2 cell culture*

Compound[b]	Expt no.	Final concn range[c] (μg/ml)	Maximal per cent CPE reduction[d]		VR[e]	Concluded virucidal effect
			Compound + 10 $CCID_{50}$ of virus	Compound + 100 $CCID_{50}$ of virus		
1	1	0.06–20.0	33	33	0.2	−
	2	0.03–20.0	17	25	0.1	−
2	1	0.0003–0.245	91	75	0.4	−
	2	0.0008–0.0245	50	50	0.2	−
3	1	2.0–625.0	58	31	0.2	−
	2	6.3–200.0	100	84	1.2	+
	3	6.3–200.0	100	69	1.5	+
4	1	6.3–625.0	58	25	0.2	−
	2	6.3–200.0	100	50	0.9	±
	3	6.3–625.0	100	100	1.6	+
5	1	20.0–2000.0	25	0	0.1	−
6	1	20.0–2000.0	25	19	0.2	−
	2	20.0–2000.0	50	0	0.3	−
	3	0.6–62.5	75	25	0.2	−
7	1	1.5–493.0	67	50	0.4	−
	2	1.5–49.3	100	94	1.0	+
	3	0.2–15.4	92	100	0.7	±
	4	0.2–15.4	100	100	1.3	+
8	1	0.006–2.0	0	0	0.0	−

[a] Compounds mixed with virus, incubated for 1 hr at 37 C prior to addition to cell culture.
[b] Compound numbers represent the compounds listed in Materials and Methods.
[c] Concentration when compound-virus mixture was in contact with cells.
[d] Only those concentrations of compound which were not completely cytotoxic were considered.
[e] VR = virus rating, a measure of CPE inhibition by a modification of the method of Ehrlich et al. (5); >1.0 = +, or possible activity; 0.5–0.9 = ±, or questionable activity; <0.5 = −, or no apparent activity.

25,000 cells/ml in Eagle's (4) basal medium (BME) was mixed with 0.05 ml of 2,000 or 20,000 cell culture 50% infectious doses ($CCID_{50}$) of vaccinia virus and poliovirus and 0.05 ml of successive 0.5 $\log_{10}$ dilutions of each compound. The BME used in this procedure was supplemented with 5% agamma calf serum and 0.5% chick embryo extract. Virus and drug had been separately incubated for 1 hr at 37 C prior to mixing. Each mixture was then pipetted into four cups in polyvinyl chloride panels. The panels were sealed with cellophane tape, incubated for 6 days at 37 C, and observed microscopically for inhibition of cytopathogenic effect (CPE). The materials and methods used for this cell culture procedure were described earlier (12).

Procedure for determining virucidal effect. The compound to be tested was dissolved in BME or in sterile physiological saline and diluted through a series of 0.5 $\log_{10}$ dilutions. Each drug solution was divided into five portions which were then mixed with equal volumes of the virus suspension and incubated for 1 hr at 37 C. The virus concentrations, prior to addition of compound, were 2,000 and 20,000 $CCID_{50}$. After incubation, the mixtures were diluted 1:100 in HEp-2 cell suspensions and pipetted into four cups in the vinyl panels. The panels were sealed, incubated at 37 C for 6 days, and examined for CPE.

Included in both antiviral and virucidal tests were virus controls (virus + medium + cells), drug cytotoxicity controls (drugs + medium + cells), and cell controls (cells + medium). Drug concentrations were selected to range from cytotoxic to nontoxic levels.

Antiviral or virucidal activity, determined by the reduction of CPE in cells infected with virus exposed to test compound, was evaluated statistically by determining a virus rating (VR); the method described by Ehrlich et al. (5) was used. One modification of this procedure was the dividing of the calculated VR by the number of cups (usually four) used for testing each dilution of test compound. In this system, a VR of 1.0 or greater was considered indicative of +, or possible activity, whereas 0.5 to 0.9 indicated ±, or questionable activity, and a figure of less than 0.5 was considered −, or indicative of no apparent activity.

Procedure for determining the virucidal effect of compounds impregnated into fabrics. Wool blanket, wool gabardine, and cotton sheeting materials were cut into four pieces approximately 46 by 60 cm. Each piece was allowed to "condition" for 24 hr in a relative humidity of 65 ± 2% at a temperature of 22 ± 2 C. A conditioned weight was taken. The compound to be used for impregnating the fabrics was diluted 1:10 in water, and each fabric piece was separately added to

TABLE 2. *Summary of studies on the antiviral[a] effect of selected compounds on vaccinia virus in HEp-2 cell culture*

Compound[b]	Expt no.	Final concn range[c] (μg/ml)	Maximal per cent CPE Reduction[d]		VR[e]	Concluded antiviral effect
			Compound + 10 $CCID_{50}$ of virus	Compound + 100 $CCID_{50}$ of virus		
1	1	0.06–20.0	0	0	0.0	−
	2	0.63–20.0	17	0	<0.1	−
2	1	0.0003–0.245	50	50	0.2	−
	2	0.0008–0.0245	42	0	0.1	−
3	1	0.02–6.25	50	25	0.2	−
4	1	0.06–6.25	0	0	0.0	−
5	1	0.2–20.0	54	0	0.1	−
6	1	0.2–20.0	41	0	0.1	−
7	1	1.5–493.0	0	0	0.0	−
	2	1.5–49.3	25	0	0.1	−
8	1	0.006–2.0	0	0	0.0	−

[a] Virus was mixed with cells, and then the compound was added. The mixture was incubated at 37 C, and inhibition of CPE was noted 6 days later.

[b] Compound numbers represent the compounds listed in Materials and Methods.

[c] Concentration when compound was in contact with cells and virus.

[d] Only those concentrations of compound which were not completely cytotoxic were considered.

[e] VR = virus rating, a measure of CPE inhibition by a modification of the method of Ehrlich et al. (5); >1.0 = +, or possible activity; 0.5–0.9 = ±, or questionable activity; <0.5 = −, or no apparent activity.

the solution. When adequately soaked, each piece was processed through a Butterworth padder with the use of a pressure of ca. 0.84 kg/cm². The fabric was then soaked again in a second bath of compound and processed through the padder again. Each was dried in an air-circulating oven at 93 C until dry. After allowing the samples to condition overnight again, a second weight was taken and the percentage of add-on was calculated from the weight increased.

TABLE 3. *Data on the impregnation of fabrics with a quaternary ammonium compound, n-alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride*

Fabric type	Avg original[a] conditioned wt (g)	Avg wet[b] wt (g)	Percentage of wet pick-up	Avg final[c] conditioned wt (g)	Avg percentage of add-on
Wool blanket	89.5	153.9	72	92.4	3.3
Wool gabardine	61.3	122.2	99	65.9	7.6
Cotton sheeting	33.4	66.6	98	34.9	4.4

[a] Average weight of five samples after being held in 65% relative humidity at 22 C for 24 hr.

[b] Average weight of four samples after processing through compound and padder twice.

[c] Average weight of four dry samples after the impregnation process.

Each treated fabric was cut into swatches 5.08 cm (2 inches) in diameter, sterilized with ethylene oxide, and exposed to the virus by pipetting 0.4 ml (ca. 10^9 $CCID_{50}$) of the virus suspension onto each swatch. The swatches were stored in 35 or 78% relative humidity, and the titer of virus on five swatches from each humidity was determined at time intervals starting from time zero in increments depending on the lengths of viral persistence on previous run control fabrics (12). Virus titers of "control" fabrics were determined concomitantly with the treated fabrics. These control fabrics were of the same type but had not been chemically treated, and were exposed to the same virus suspension as the treated fabrics. Sterile swatches of each treated fabric were tested to determine their toxicity in cell culture.

More detailed descriptions of the materials and procedures for determining viral persistence were described previously (2, 12).

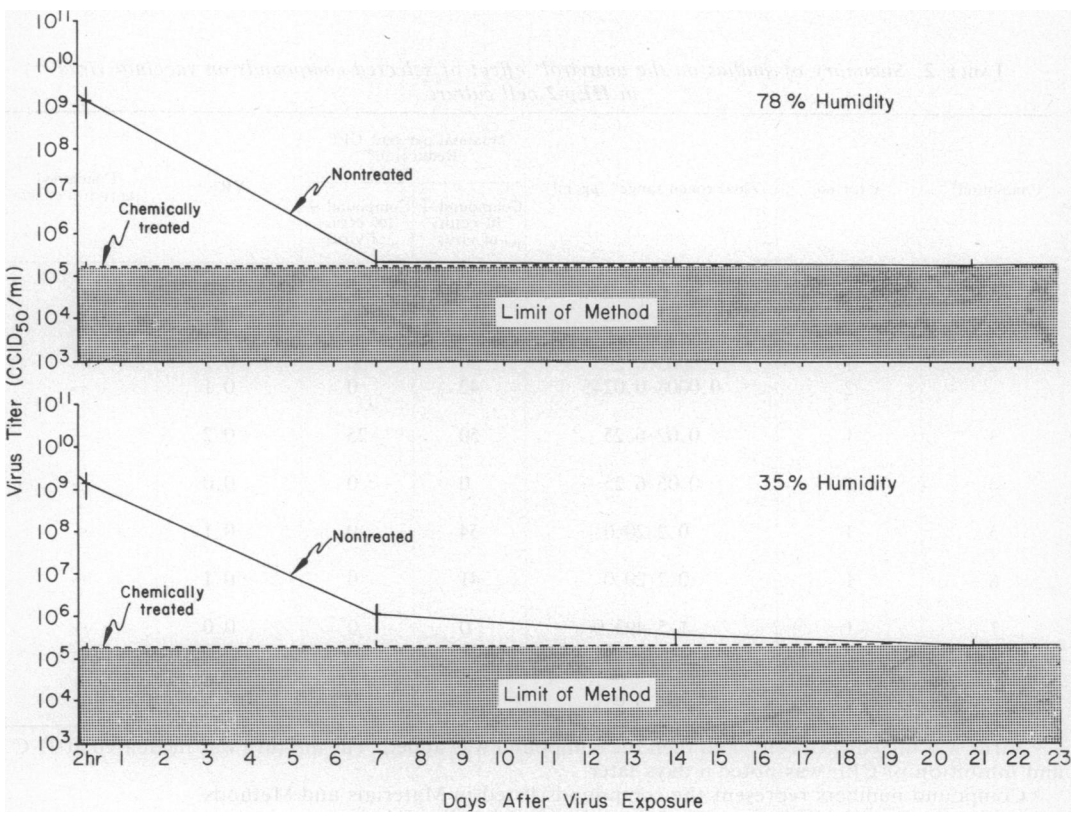

FIG. 1. *Persistence of vaccinia virus on wool blanketing material impregnated with n-alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride and held at 25 C in two humidities. Data expressed as mean virus titers ± 95% confidence limits at each time tested.*

RESULTS

A total of 60 experiments were carried out to determine the antiviral and virucidal effect of the eight test compounds. The results of these studies with vaccinia virus are summarized in Tables 1 and 2, respectively. Definite virucidal activity was observed in vaccinia virus experiments with the quaternary ammonium compounds no. 3, 4, and 7 (Table 1). Suggestive antiviral activity against vaccinia virus was observed in several experiments, but statistical analysis indicated this activity to be insignificant (Table 2). None of the compounds was active against poliovirus.

Since compound 3 had significant virucidal activity against vaccinia virus, this compound was used to impregnate the wool and cotton fabrics. The data on the impregnation of these fabrics are summarized in Table 3. Studies were carried out to determine whether the time of viral persistence was decreased on the impregnated materials as compared with the duration of viral persistence on similar nonimpregnated materials tested concomitantly. The results of the study are shown in Fig. 1–3. No virus could be recovered at any time from the impregnated fabrics, although the duration of viral persistence on the nonimpregnated fabrics varied from 5 to 21 days. Thus, the chemical treatment resulted in an almost immediate reduction of more than 10^4 infectious viral units.

An experiment was carried out to determine the persistence of vaccinia virus and poliovirus on cotton wash-and-wear fabric exposed to the viruses by direct contact. The results of these experiments (Fig. 4) indicate that vaccinia virus persists no longer than 1 day postexposure, regardless of humidity. The poliovirus persisted approximately 3 to 4 days in detectable quantities.

DISCUSSION

Fabric self-sanitizing is not a modern concept. Early Egyptians used herbs and spices to preserve the fabrics used to wrap their mummies. Cedar oil was apparently utilized by the Romans as a preservative, and quaternary ammonium compounds were used effectively to treat fabrics worn by German troops during World War II (7). Modern-day fabric treatments with germicides have been attempted for a number of purposes: to control the dissemination of infectious agents, to reduce bacteria-induced odors, to prolong the life of fabrics, to prevent staining by microorganisms, and for imparting a contaminant-free environment to surgical dressings. The use of such a self-sanitizing process for rendering fabrics refractory to viruses, however, has virtually never been attempted.

Viruses are apparently susceptible to inactivation by a number of chemical substances, as re-

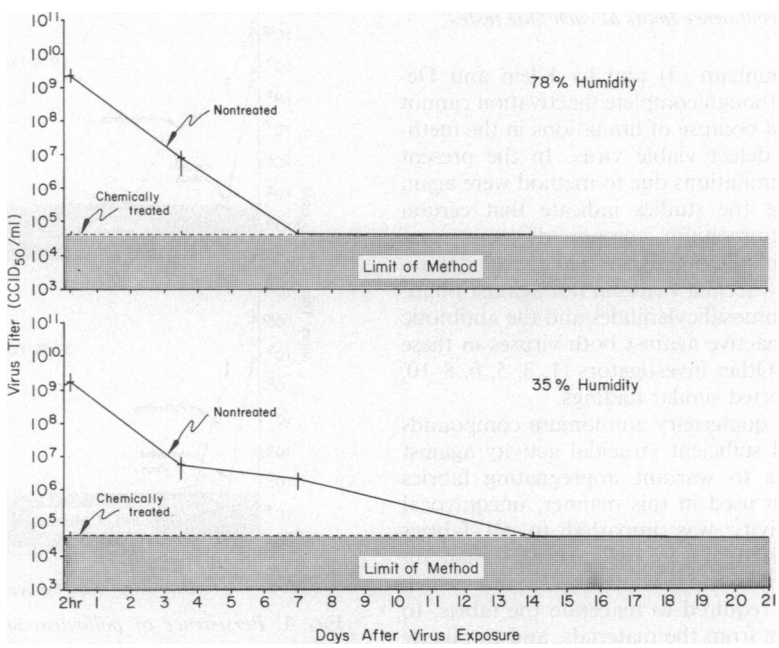

FIG. 2. *Persistence of vaccinia virus on wool gabardine material impregnated with n-alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride and held at 25 C in two humidities. Data expressed as mean virus titers ± 95% confidence limits at each time tested.*

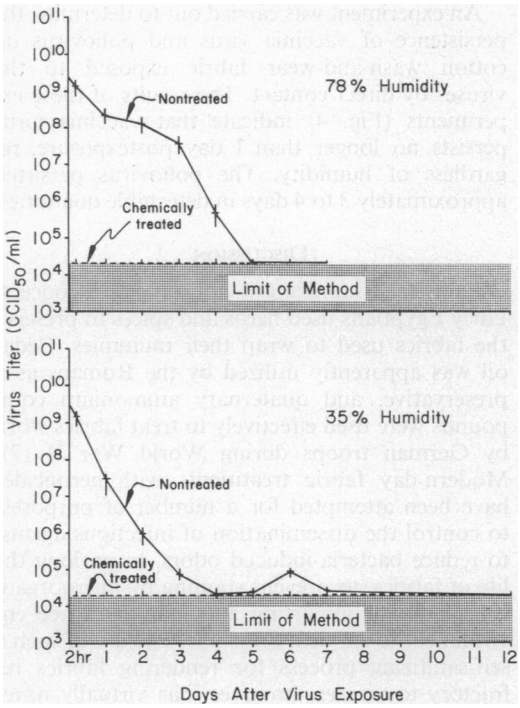

FIG. 3. *Persistence of vaccinia virus on cotton sheeting material impregnated with n-alkyl (C14, C12, C16) dimethyl benzyl ammonium chloride and held at 25 C in two humidities. Data expressed as mean virus titers ± 95% confidence limits at each time tested.*

viewed by Dunham (3) and by Klein and DeForest (9), although complete inactivation cannot be determined because of limitations in the methods used to detect viable virus. In the present study, these limitations due to method were again apparent, but the studies indicate that certain quaternary ammonium compounds now commercially available apparently have virucidal activity against vaccinia virus but not against poliovirus. The bromosalicylanilides and the antibiotic tested were inactive against both viruses in these experiments. Other investigators (1, 3, 5, 6, 8–10, 13) have reported similar findings.

One of the quaternary ammonium compounds demonstrated sufficient virucidal activity against vaccinia virus to warrant impregnating fabrics with it. When used in this manner, unequivocal virucidal activity was imparted to all fabrics studied. Virus titers were reduced more than 10^4 on each fabric in approximately 30 min, which was the time required to macerate the fabric, to elute the virus from the materials, and to pipette the eluted agent into the indicator cell culture system.

The differences in viral persistence on the untreated fabrics essentially confirmed the findings reported previously (12), in which vaccinia virus persisted longer on wool fabrics than on cotton fabrics, whether stored in 35 or 78% humidity. The viral persistence on the nonimpregnated wool gabardine material was of lesser duration than reported previously, but a new fabric, which was noticeably different in color and texture, was used in the present study.

A manufacturer's modified triazone finish applied to cotton material to impart a wash-and-wear characteristic to the fabric apparently may also have been effective in reducing the persistence of both poliovirus and vaccinia virus, since viral persistence on this fabric was less than 1 day, compared to a minimum of 5 days on other untreated cotton fabric types (2, 12). Since no comparative experiments were carried out with a similar fabric which had not received the wash-and-wear finish, however, actual comparisons cannot be made.

The results of these studies with vaccinia virus suggest that treatments of fabrics to render them self-sanitizing against that virus may be of practical use, particularly for materials to be used in areas suspected of being contaminated by the agent. If effective chemicals can be found which have significant activity against other viruses, such a process may have broad application.

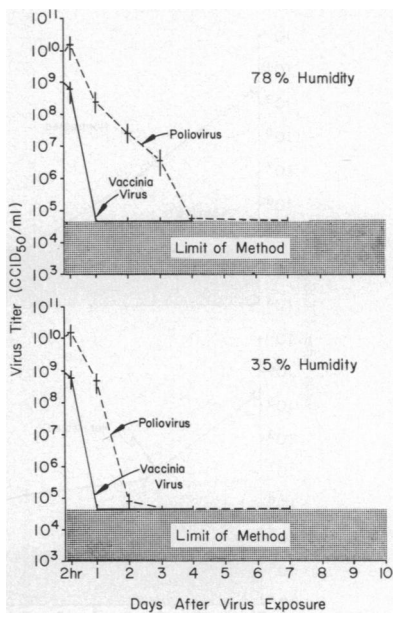

FIG. 4. *Persistence of poliovirus and vaccinia virus pipetted on a cotton fabric impregnated with a wash-and-wear finish. The virus-exposed fabric was held at 25 C in two humidities. Data expressed as mean virus titer ± 95% confidence limits at each time tested.*

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