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ANTIVIRAL ACTIVITY OF AN ALCOHOLIC HAND DISINFECTANT. COMPARISON OF THE IN VITRO SUSPENSION TEST WITH IN VIVO EXPERIMENTS ON HANDS, AND ON INDIVIDUAL FINGERTIPS*

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The inactivation of eleven viruses by a commercial alcoholic hand disinfectant ('Desderman') was tested by three different procedures, viz., in vitro in a standardized suspension test, in vivo on the entire surface of both hands, and in vivo on individual fingertips of a single volunteer. The test protocols were chosen such as to make results comparable. The influence of varying disinfectant/virus volume ratios, serum protein loads, and reaction temperatures was evaluated in vitro, and partly demonstrated in vivo. In some experiments, 5% formaldehyde was included as a reference disinfectant. The experiments support the prevailing concept, that enveloped viruses are considerably more susceptible to alcoholic disinfection than naked ones. Additionally, enveloped viruses were shown to be subject to more 'spontaneous' decay on skin. The main result of the study appears to be that the in vitro model predicted a greater effectiveness of the disinfectant than was observed in vivo both in the 'hand' test and the 'finger' test, although some differences were noted between these tests. Reasons for the lesser in vivo inactivation may lie in the evaporation of the disinfectant with ensuing changes in disinfectant/virus volume ratio, effective temperature, and inactivation time, all shown to influence disinfection.

Other mechanisms such as a 'sheltering' effect of the skin may have also been operating. The results suggest that, at the present stand of knowledge, in vitro screening tests should be complemented by in vivo tests for the evaluation of antiviral hand disinfectants.

antiviral disinfection; alcoholic disinfectants; skin disinfection; suspension test

INTRODUCTION

Disinfection has been the domain of bacteriologists almost exclusively for a long period of time. In recent years, however, the antiviral aspects of disinfection met with an increasing interest stimulated by practical needs of hygiene in veterinary and human medicine. Mainly such chemicals have been tested so far that were already known to be active against bacteria. Available results indicate that viruses vary considerably in their susceptibility to chemical disinfection, probably more so than bacteria do.

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In an attempt to standardize the evaluation of antiviral chemical disinfectants, an in vitro procedure has been proposed by a committee of experts [2]. In this 'suspension test' the kinetics of inactivation of virus particles suspended in dilutions of disinfectant are determined. Conditions of temperature, disinfectant concentration, and serum protein added (to simulate interference by organic matter), are controlled. A disinfectant is classified as 'effective against viruses' if it inactivates the infectivity of four representative viruses (poliovirus 1, strain Mahoney; vacciniavirus, strain Elstree; adenovirus type 2, strain Adenoid 6; and simian virus 40 (SV 40), strain 777) by four $\log_{10}$ within two min (hand disinfectants), or 60 min (other disinfectants) [2].

In this approach, the suspension test is used as a model for a number of disinfection situations, including hand disinfection. To what extent and under what conditions the in vitro results may be considered representative for the hand disinfection situation, has so far not been determined experimentally.

In the present study, the performance of a commercial alcoholic hand disinfectant ('Desderman®') in the suspension test was compared with its effectivity on artificially virus contaminated hands of a volunteer under standardized conditions. Analogous experiments were carried out using individual fingertips of the volunteer: it was thus aimed to establish an in vivo hand disinfectant evaluation procedure with a reduced number of volunteers needed.

MATERIALS AND METHODS

Outline of experiments

Inactivation of viruses by 'Desderman' was tested under standard conditions in vitro (suspension test), on the entire surface of both hands (hand test), and on individual fingertips (finger test). A total of eleven viruses was used, special weight being given to the enterovirus group. The same person volunteered in all in vivo experiments. The test protocols were designed such as to make results directly comparable. The relative influence of some parameters in the suspension test was evaluated, and the effect of temperature was demonstrated in vivo. Formaldehyde (5% w/v) served as a reference disinfectant.

Disinfectants

Desderman is a commercial alcoholic hand disinfectant containing 78.2% (w/w) of 95.3% (v/v) ethanol, 10% (v/v) isopropanol, and 0.1% (w/w) 2,3,4,5-tetrabrome-6-methylphenol. Aliquots of lots 0059 and 0175 were provided by the producer (Schülke u. Mayr, Norderstedt, F.R.G.). Formaldehyde (Merck, Darmstadt, F.R.G.) was titrated using hydroxylammoniumchloride (Merck), and adjusted to 5% (w/v) and pH 7.

Cell cultures

GMK, a continuous epithelial cell line derived from African green monkey kidney tissue [12], was used in most experiments. HeLa cells were a gift from R. Wigand, Hom-

burg, F.R.G. CV-1 cells were obtained through the courtesy of A. Grässmann, Berlin, F.R.G. Primary chick embryo fibroblasts (CEF) were cultured from embryonated eggs, 9–13 days old, purchased from a local poultry farm. Cells were grown in Eagle's minimum essential medium (MEM; Flow Laboratories, Meckenheim, F.R.G.) enriched with 5% fetal bovine serum (FBS; Flow, or Seromed, Munich, F.R.G.). HeLa cells were grown in the presence of 5% newborn calf serum (NCS; Seromed).

Virus

Fowl plague virus (FPV) and influenza virus A/WSN were a gift from H.D. Klenk, Giessen, F.R.G. After one passage in 11-day-old chick embryos in this laboratory, allantoic fluids cleared by centrifugation were used as crude virus suspensions. Vaccinia virus, strain MVA, was a gift from H.A. Stickl, Munich, F.R.G. The virus was passaged twice in CEF and harvested as cell-associated virus. Infected cells were ultrasonicated and taken up in a small volume of medium to yield crude virus suspension. Adenovirus type 5, a gift from R. Wigand, was passaged three times in HeLa cells in this laboratory. Poliovirus type 1 (Mahoney), type 2 (MEF1), and type 3 (Saukett) were described previously [10]. These viruses had a history of monkey kidney and HeLa cell passages and were grown in MEM with 5% NCS. One lot of poliovirus 1 was grown without serum added, another in the presence of 1% FBS, but harvested as cell-associated virus. Coxsackievirus B3 (Nancy) was described before [10]; of coxsackievirus B4, the a^s particle type was used [4]. Echo 9, strains Hill and Barty, were described previously [9,26]. Five strains of Echo 11 (Gregory, Porz, Aus I, Aus II, and U) were provided by Th. Mertens [21]. Coxsackie- and echoviruses were grown in GMK cells with MEM containing 5 or 2% fetal or newborn calf serum. SV40 was obtained from A. Grässmann, Berlin, F.R.G., and had one passage in CV-1 cells with MEM 5% FBS in this laboratory. Crude suspensions of all naked viruses were obtained through freeze-thawing of culture flasks when 75–100% of cells showed cytopathic changes, followed by ultrasonication of cells in their media. Enterovirus suspensions were stored at -25°C, all other viruses at -70°C.

Plaque assay

For the determination of infectivity titers the plaque assay was used throughout. Variable parts of the assay, which was based on previously published procedures [4,10, 11], are summarized in Table 1; constant parts were as follows. Plastic petri dishes 60 mm in diameter (Greiner, Nürtingen, F.R.G.) with confluent cell monolayers were washed once with 2 ml phosphate-buffered saline (PBS) [8]. Two dishes per dilution were inoculated with 0.2 ml of serial 10-fold dilutions of virus prepared on crushed ice. After an adsorption period of 1 h during which the petri dishes were gently moved several times to spread the inoculum over the entire cell surface area, the inoculum was removed, the cells were washed once with PBS, and 5 ml overlay per dish was added. 100 volume parts of overlay consisted of 50 parts of 1.8% (w/v) agar or 1.5% (w/v) agarose (Serva, Heidelberg, F.R.G.) which were mixed with 49 or 45 parts of Dulbecco's modified MEM in double strength (Flow), and 1 and 5 parts, respectively, of serum. Overlay for adenovirus

TABLE 1

Variable parts of the plaque assay

Virus	Cell monolayers	Dilution of virus in	Adsorption temperature	Overlay	Further overlays	NR[b] staining on day	Read (without NR) on day
FPV Influenza A/WSN Vaccinia MVA	CEF	PBS, 1% NCS	37°C	agarose, 1% NCS	no	4	–
Adeno 5	HeLa	PBS, 1% NCS	37°C	agarose, 1% NCS + 26.7 mM $MgCl_2$	yes	14 (1st exp.)	8 (2nd exp.)
Polio 1–3	GMK	PBS	RT[a]	agar, 1% NCS	no	–	2–3
Coxsackie B3,4 Echo 9,11	GMK	PBS	RT	agarose, 1% NCS	no	–	2–6
SV 40	CV-1	PBS, 1% NCS	37°C	agarose, 5% FBS	yes	13	–

[a] RT, room temperature (22–26°C).
[b] NR, neutral red.

5 titrations was enriched with 26.7 mM $MgCl_2$ (R. Wigand, personal communication). The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air; in some instances further nutrient overlays were added. Plaques were counted either 4–16 h after staining of cultures with 2 ml 0.01% neutral red in 0.9% agar, or without staining. Plaque titers were recorded as the decadic logarithms of the number of plaque forming units (pfu) per 0.2 ml titrated virus suspension.

Suspension test

The method proposed in Ref. 2 was used with only slight modifications. Unless otherwise stated, experiments were carried out in a water bath of 20± 0.1°C with reactants adapted to this temperature. Crude virus suspension (0.2 ml) was mixed with 0.1 ml FBS in a test tube. The inactivation time was started when 2.7 ml disinfectant was added and mixed quickly on a vortex mixer. At 2-min intervals samples (0.2 ml) were drawn, and diluted immediately on ice in PBS with or without 1% NCS for plaque titration (cf. Table 1). If viruses required inactivation times shorter than 2 min, individual reaction mixtures were tested at each time point. A virus control was included using distilled water instead of disinfectant. Titrations at the beginning and the end of the test did not, in any case, show spontaneous decay of the control virus. Therefore, the mean of the two titrations was recorded as the virus titer at time zero. Titer reductions were calculated by subtracting the virus titers at experimental time points from this value.

Cytotoxic effects of the disinfectants

Such effects were controlled by the following two-step-procedure included in each suspension test. A test mixture of disinfectant, FBS, and water was prepared as in the main test, virus being replaced by water. 10-fold dilutions of this mixture were 'inoculated' in cell cultures, two per dilution, 0.2 ml per petri dish. After an 'adsorption period' as in the plaque assay, the disinfectant dilution was removed and the cells were washed with 2 ml PBS. Then the cultures were infected uniformly, depending upon expected plaque size, with 10–100 pfu of virus in 0.2 ml. Adsorption and further proceedings were those of the plaque assay. The reciprocal of the lowest dilution of disinfectant that did *not* interfere with the normal development of plaques indicated the lowest virus concentration detectable under conditions of disinfectant present; its $\log_{10}$ was recorded as the 'level of sensitivity'.

Parameters influencing the suspension test

(a) Disinfectant/virus volume ratio. The volume ratio of disinfectant and virus, usually 27:2 (see above under Suspension Test), was changed into 23:6, 19:10, and 15:14; i.e., progressively more crude virus suspension was exposed to less disinfectant. Deviating from standard procedure, the test with FPV was carried out at room temperature (25°C).

(b) Serum protein load. A modified reaction mixture was used: 10 or 20% of the dis-

infectant volume of the standard suspension test was replaced by FBS, or water as a control.

(c) Reaction temperature. Suspension tests were carried out at 0°C on crushed ice, and at 20 and 26°C in a water bath. FPV was tested at 0°C and at room temperature (25°C).

(d) Virus type and strain differences. At least two serologic types of each enterovirus group, and 2 and 5 strains of echoviruses 9 and 11, respectively, were submitted to the standard suspension test.

Hand test

A control run with water and a test run with disinfectant were carried out on the same day. The volunteer washed his hands under hand-warm running tap water with ordinary soap for 5 min and dried them twice with two paper towels. Crude virus suspension (0.5 ml) was pipetted on the palm of his left hand and distributed by rubbing movements similar to those in handwashing over the surfaces of both hands, excluding the wrists. This took 90 s, followed by a 30-s pause to allow for drying. The following 10-min inactivation period was divided into five 2-min intervals. In each interval 1.4 ml water (control run) or disinfectant (test run) was pipetted on the palm of the left hand, rubbed in with washing movements including the wrists for 1 min, and the remaining time was allowed to elapse without rubbing. After 10 min, the hands were washed for 5 min in a 20-cm petri dish on crushed ice, containing 50 ml PBS with 3% NCS, or PBS alone (enteroviruses). The original virus suspension as well as aliquots of the washing fluids of control and test runs were kept frozen waiting titration. From the titer of the original material and the dilution of virus (0.5 ml in 50 ml washing fluid: dilution factor 1:100), the sample titer was calculated that would have been expected had recovery in the virus control been complete.

Finger test

The volunteer washed his hands as described above. He folded his hands in such a way that the distal finger pads of homologous fingers of right and left hand were facing each other and in touch. Into the capillary cleft between opposing fingers 10 µl crude virus suspension was pipetted and rubbed in gently for 90 s. Hands were separated for another 30 s to allow for drying. The following 10-min inactivation period was divided into five 2-min intervals. In each interval 30 µl water (control) or disinfectant (test) was pipetted onto pairs of fingertips in a random distribution, and rubbed in with gentle movements for 1 min. The remaining time was allowed to elapse without rubbing. Since water and formaldehyde evaporated to a lesser extent than 'Desderman', no water and formaldehyde, respectively, were applied at 4 and 8 min for fear of dropping liquid. After 10 min, samples from individual fingers were taken by rubbing the finger pads with ten brisk strokes on the ground of 6-cm plastic petri dishes, containing 2 ml ice-cold PBS, 3% NCS, or PBS alone, two samples each of control (water) and test (disinfectant). The

samples were stored frozen and titrated together with the original virus material. The mean titers of control and test as well as the expected titer of the virus control assuming complete recovery, were calculated. The scatter of sample titers had been determined with poliovirus 1 using all fingers as virus control: the mean titer ± S.D. had been 5.0 ± 0.4, the range being 4.6–5.6. Preliminary experiments had also shown that a smaller amount of virus – 1 µl applied to two fingers – resulted in considerably larger scatter of titers.

Temperature changes of evaporating ethanol in vitro

Two glass petri dishes (internal diameter 18.5 cm) containing 200 ml 70% (v/v) ethanol, one uncovered and the other covered with plastic foil, were stirred on a magnetic stirrer and exposed to the air stream of an electric room ventilator. The temperatures of the ethanol were recorded at 2-min intervals.

Influence of temperature on disinfection in vivo.

A modified finger test was carried out in a constant temperature room of 4°C. Three 10-ml volumes of 'Desderman' in 90-mm plastic petri dishes were adapted in three water baths to 5°C, 20°C, and 30°C. After handwashing as described above, virus (10 µl per two fingers) was applied to the fingertips, excluding thumbs, and rubbed in as described above. The four fingertips of one hand, randomly attributed to a temperature, were immersed in the disinfectant for 5 min and moved with minimal up-and-downward movements in a 3-s rhythm. The fingers were tipped on a thick layer of absorbing paper tissue (Repellex; Temca Chemische Union, Nürnberg, F.R.G.) to remove excessive 'Desderman', and samples from individual fingertips were taken as described above. Two hands (8 fingers) were used at each temperature, however, one sample at each temperature was lost due to an experimental error.

RESULTS

Standard suspension test

The inactivation kinetics of eleven viruses under standard conditions are given in Fig. 1. As a reference, the inactivation of poliovirus 1 by 5% formaldehyde is included. Whereas the titers of the three enveloped viruses (top row) were reduced to undetectable levels within 30 s, naked viruses showed a slower, biphasic inactivation pattern resulting in 2.0–4.5 $\log_{10}$ titer reduction at 10 minutes.

Parameters influencing the standard suspension test

(a) Disinfectant/virus volume ratio. The results are summarized in Fig. 2. Decreasing the volume ratio to 15:14 did not demonstrably influence the action of 'Desderman' on FPV, which appeared to be overwhelmingly susceptible to the disinfectant. The inactivation of poliovirus 1, however, was increasingly inhibited; practically no effect was seen at a volume ratio 15:14 and 19:10.

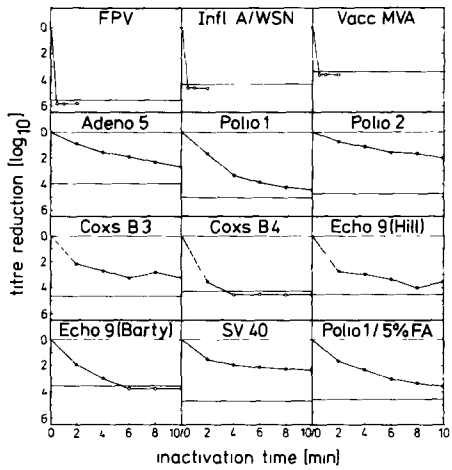

Fig. 1. Inactivation of eleven viruses by 'Desderman' in the suspension test under standard conditions. Included, as a reference, the inactivation of poliovirus 1 by 5% formaldehyde. Open symbols: titre reduction below level of sensitivity.

(b) Serum protein load. The effect of an additional serum protein load on the inactivation of poliovirus 1 is summarized in Fig. 3. Replacing 10 or 20% of the disinfectant volume by water did not apparently impair the activity of both 'Desderman' and 5% formaldehyde (controls; cf. Fig. 1). Replacement by FBS led to a marked, concentration-dependent inhibition of 'Desderman'; formaldehyde, however, was not influenced by the increased serum load.

(c) Reaction temperature. The reaction temperature had a varying influence on inactivation kinetics, depending upon virus and disinfectant used. The inactivation of FPV by 'Desderman' was, at 0°C, equally effective as at 25°C (titer reduction to undetectable over 4.9 $\log_{10}$ within 30 s in both cases; no figure shown). The action of 'Desderman' on poliovirus 1 was strongly, that of formaldehyde on the same virus less temperature-

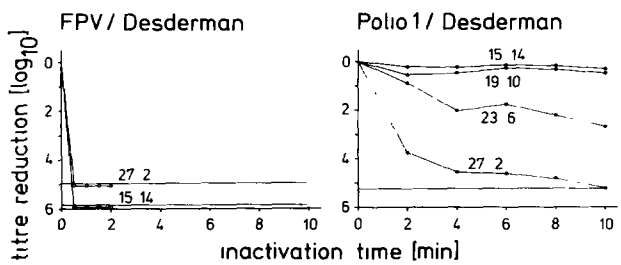

Fig. 2. Influence of different disinfectant/virus volume ratios on the inactivation of fowl plague virus (FPV) and poliovirus 1 by 'Desderman'.

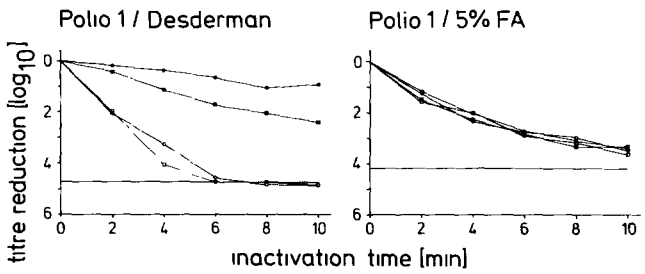

Fig. 3. Influence of serum protein added on the inactivation of poliovirus 1 by 'Desderman', and by 5% formaldehyde. 10% (squares), or 20% (circles) of the disinfectant volume were replaced by fetal bovine serum (closed symbols), or water as a control (open symbols).

dependent (Fig. 4). The disinfecting property of 'Desderman' on poliovirus 1 was apparently abolished at 0°C.

(d) Virus type and strain differences. The effectiveness of 'Desderman' against enteroviruses varied with serologic type, and, to a lesser extent, with strains within a type (Figs. 1 and 5). The largest difference was seen between poliovirus 1 and 2, poliovirus 3 (data not shown) behaving similar to poliovirus 1. Differences between two echovirus 9 strains (Fig. 1), and five echovirus 11 strains (Fig. 5) were less prominent. Strains Porz and Aus I appeared to be more resistant than the other echovirus 11 strains.

Hand test

The results of the hand test are summarized in Table 2. The same pattern as in the suspension test emerged: the enveloped viruses were reduced to undetectable levels, whereas the naked viruses were still detectable in varying titers after disinfection. As to the water controls of the naked viruses, a considerably smaller fraction of adenovirus 5 and echovirus 9 Hill was recovered than of the other viruses. Different mechanisms such

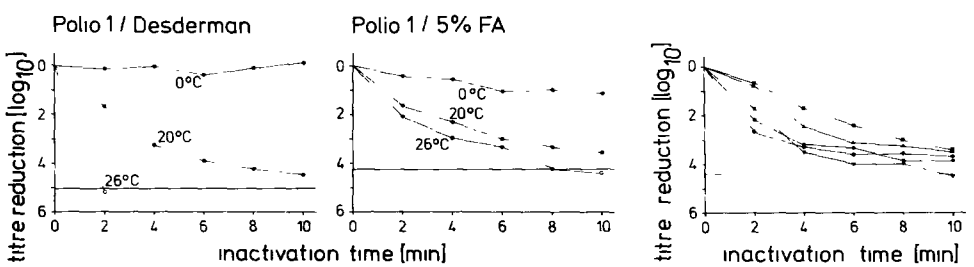

Fig. 4. Influence of the reaction temperature on the inactivation of poliovirus 1 by 'Desderman', and 5% formaldehyde.

Fig. 5. Inactivation of five echovirus 11 strains by 'Desderman'. ■, Porz; ▲, Aus I; ◆, U; ●, Gregory; ▼, Aus II.

TABLE 2

Hand test: inactivation of eleven viruses by 'Desderman'

Virus	Sample titers (in $\log_{10}$/0.2 ml)			Titer reduction control-test (in $\log_{10}$)
	Expected[b]	Control[c]	Test[d]	
FPV	6.2	2.5	<0	>2.5
Influenza A/WSN	5.5	2.5	<0	>2.5
Vaccinia MVA	4.4	1.4	<0	>1.4
Adeno 5	5.1	2.2	0.1	2.1
Polio 1	6.5	5.4	4.4	1.0
Polio 2	5.2	3.7	3.5	0.2
Coxsackie B3	6.0	4.3	3.2	1.1
Coxsackie B4	5.9	4.2	2.9	1.3
Echo 9 Hill	6.1	2.8	2.1	0.7
Echo 9 Barty	5.3	4.3	3.0	1.3
SV 40	5.9	3.9	3.0	0.9
Polio 1/5% FA[a]	6.8	5.7	<0	>5.7

[a] 5% formaldehyde was included as a reference disinfectant.
[b] Amount of virus applied to hands.
[c] Amount of virus actually recovered from hands treated with water.
[d] Amount of virus recovered from hands treated with disinfectant.

as a higher 'spontaneous decay' of virus on skin (discussed below), or a greater mechanical adherence of the virus particles to skin are feasible as an explanation for this remarkable finding. Nonetheless, the disinfectant action could be quantitated in all naked viruses, and was about one $\log_{10}$ greater in adenovirus 5 than in any other naked virus tested. The disinfectant-specific inactivation of the enveloped viruses, however, could only be observed within a small log range, as virus infectivity was also markedly reduced in the water control. Formaldehyde inactivated poliovirus 1 by over 5 $\log_{10}$ to undetectable levels.

Finger test

The results of this test are summarized in Table 3. The pattern of inactivation of naked and enveloped viruses was apparently the same as in the hand test. However, the enveloped viruses were already reduced to undetectable levels in the water controls, thus a disinfectant-specific inactivation could not be demonstrated. Adenovirus 5 and echovirus 9 Hill were again recovered in lower titers in the virus controls than the other naked viruses, which again may hint at a greater 'spontaneous decay', or a greater adherence of these viruses on skin. This time, adenovirus 5 was reduced by 'Desderman' to an undetectable level, the observable titer reduction being at least 2.3 $\log_{10}$. All other naked viruses could be recovered after disinfection, but the titer reductions were, on the average, about one $\log_{10}$ greater than in the hand test. Formaldehyde inactivated poliovirus 1 by over 4 $\log_{10}$ to undetectable levels.

TABLE 3

Finger test: inactivation of eleven viruses by 'Desderman'

Virus	Sample titers (in $\log_{10}$/0.2 ml)			Titer reduction control-test (in $\log_{10}$)
	Expected	Control	Test	
FPV	6.1	<0	<0	–
Influenza A/WSN	5.0	<0	<0	–
Vaccinia MVA	4.0	<0	<0	–
Adeno 5	4.5	2.3	<0	>2.3
Polio 1	5.5	4.4	1.9	2.5
Polio 2	5.5	4.1	3.4	0.7
Coxsackie B3	5.6	4.5	1.6	2.9
Coxsackie B4	4.9	4.8	1.8	3.0
Echo 9 Hill	5.1	2.7	0.1	2.6
Echo 9 Barty	5.6	4.9	2.7	2.2
SV 40	5.3	3.8	2.0	1.8
Polio 1/5% FA[a]	6.2	4.3	<0	>4.3

[a] 5% formaldehyde was included as a reference disinfectant.

Correlation of suspension test and finger test with hand test

In fig. 6 the titer reductions of the naked viruses in suspension test (10-min values) and finger test, were plotted against the respective values of the hand test. For reasons discussed below, adenovirus 5 was not included. Gathering of experimental points above the broken lines indicates that inactivation in suspension test and finger test was greater than in the hand test. Also, inactivation was greater in the suspension test than in the finger test. A weak, but definite correlation between titer reductions in the different tests was found.

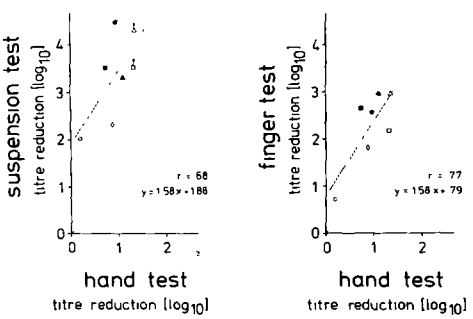

Fig. 6. Correlation of inactivation of the naked viruses (except adenovirus 5) in suspension test and finger test, with that in the hand test. ●, poliovirus 1; ○, poliovirus 2; ▲, coxsackievirus B3; △, coxsackievirus B4; ■, echovirus 9 (Barty); □, echovirus 9 (Hill); ◇, simian virus 40.

Temperature changes of evaporating ethanol in vitro

Evaporating 70% ethanol exerted a considerable cooling effect upon the liquid phase. While the temperature of ethanol in the covered control dish remained constant (25°C, ambient temperature), that of the uncovered petri dish fell to 19°C in 2 min, to 14°C in 8 min, and reached a plateau at 13°C after about 15 min.

Influence of temperature on disinfection in vivo

Exposing poliovirus 1 on fingertips to 'Desderman' of varying temperature yielded the following mean sample titers and titer ranges: 5°C, 3.1 (range 2.2–3.8); 20°C, 1.7 (range 1.3–2.4); 30°C, 1.3 (range 0.2–2.4). The sample titers at 20°C and 30°C were significantly lower than at 5°C; the difference between 20°C and 30°C was not significant ($P < 0.01$, U-test of Wilcoxon, Mann and Whitney [22]).

DISCUSSION

In the suspension test with 'Desderman', the viruses tested fell – as to be expected – into two major groups which agree with the morphological classes of enveloped and naked viruses. The enveloped viruses were completely inactivated within the shortest time tested. The effect of 'Desderman' on FPV was so overwhelming that it could not be influenced by varying conditions of temperature or disinfectant/virus volume ratio within the scope of our experiments. On the other hand, naked viruses were inactivated slowly, and inactivation was incomplete at 10 min in most cases. Furthermore, the inactivation of poliovirus 1 was subject to considerable influences of temperature, disinfectant/virus volume ratio, and protein load. Inferring from poliovirus 1, one may say that the action of 'Desderman' on naked viruses can be effective, when circumstances are favorable. The action of formaldehyde appears to be slow but steady, and less influenced by external factors such as temperature or protein load.

'Desderman', like formaldehyde, produced biphasic inactivation curves with a faster initial, and a slower ensuing phase. Such a kinetic pattern has been interpreted, in the case of formaldehyde, as evidence that both the capsid protein shell and the nucleic acid of a virion are subject to alteration during inactivation [14].

Within the group of naked viruses, differences were not only observed between serologic types (especially poliovirus types 1 and 2), but also minor differences between strains of a serologic type (echovirus 11). Resistance to chemical inactivation tested by a method similar to the suspension test may turn out to be useful as an intraserotypic marker of field isolates.

The in vitro disinfection of viruses has been investigated by a number of workers using protocols that varied considerably in reaction temperature, disinfectant/virus volume ratio, protein load, and disinfectants used [1,3,5–7,13,18,20,24,25]. The variation in protocols makes interpreting results and comparing disinfectants difficult. Efforts to standardize antiviral disinfectant testing [2,19,27] should therefore be welcome. As far as alcoholic or surface-active disinfectants have been tested [1,5–7,18,24], previous

experience indicating that viruses fall into two groups of highly different susceptibility, is supported by our results. Two discrepancies between previous and our results, however, were noted: Adenovirus 2, a naked virus, was reported [18] to be inactivated in vitro similar to enveloped viruses and was therefore counted with these among the 'lipophilic' viruses, whereas in our in vitro system, adenovirus 5 showed an inactivation pattern similar to that of other naked viruses, e.g., poliovirus 2. In our in vivo tests, however, adenovirus 5 exhibited a somewhat intermediate behavior with both considerable 'spontaneous decay', and a greater susceptibility to 'Desderman' than the other naked viruses (hand test). Accordingly, adenovirus 5 was not included in the correlations of Fig. 6. Secondly, rotavirus SA 11 was reported [1] to be inactivated by 'Desderman' by about 3 $\log_{10}$ – to undetectable levels – within 1 min. Contrary to what would be inferred from our results with the naked virus poliovirus 1 (Fig. 3), the addition of 20% FBS to the reaction mixture did not inhibit the effect of 'Desderman' on SA 11 in that study. It may be concluded that in many instances the concept of enveloped susceptible, and naked resistant viruses may be valid as a first approximation, but exceptions are to be expected and inferences from one virus to another should only be made with caution. The disinfectant testing with 'model viruses' [2] is thus not free of inherent problems.

Survival, and disinfection, of virus on hands has been investigated by several workers [3,15–17,23]. For the transmission of rhinoviruses, hands are incriminated to play an important role. Rhinovirus in nasal secretions can be transmitted from experimentally infected to uninfected volunteers by direct hand to hand contact, and cause disease; however, transmission can be interrupted by pretreating fingers with aqueous iodine [3, 15]. The enveloped respiratory syncytial virus (RSV) was shown to survive for prolonged times on different surfaces, including skin. Survival on hands was of the order of 20–30 min, this time was shortened to an average of 15 min if the hands were used, thus allowing for contact with other surfaces [16]. In a study on the disinfection of vaccinia-virus, strain Berlin, 1.5% chloramine T and 70% isopropanol were shown to completely inactivate virus applied to thumb and forefinger of a volunteer within 2 and 5 min, respectively [23].

An interesting finding in our study was the reduction to undetectable levels of enveloped viruses in the controls of the finger test (Table 3), indicating decay attributable to factors other than the action of the disinfectant. In fact, if we compare the average differences between expected and control titers (Tables 2 and 3) of the three enveloped viruses with those of the eight naked viruses, we find that in the hand test, 1.4 $\log_{10}$ more enveloped virus is lost than naked virus; in the finger test this difference amounts to more than 3.7 $\log_{10}$. These are, of course, only relative figures, an absolute measure of 'spontaneous decay' cannot be derived from our data. As to the cause of 'spontaneous decay', one may speculate that the chemical milieu of the skin surface, e.g., pH or fatty acids, contributed to inactivating virions, predominantly those with a lipid-containing envelope. In addition, rubbing might have mechanically destroyed virions, this hypothesis being supported by the fact that subjectively more rubbing per skin surface area

occurred in the finger test than in the hand test, and enveloped viruses were more inactivated in the controls of the finger test than of the hand test. 'Spontaneous decay' appears also to be a likely explanation for the comparatively low titers of adenovirus 5 recovered in the controls of hand test and finger test, as the 'spikes' in the virion structure may render the virus susceptible to mechanical inactivation.

The significance of 'spontaneous decay', in our view, is that it marks a qualitative difference between the 'reality' of hand disinfection and its model, the suspension test. It also underlines the difference between enveloped and naked viruses in the hand disinfection situation.

The protocols of in vitro and in vivo tests were intended to make results directly comparable, using disinfectant/virus volume ratios of approximately 1:14 and inactivation times of 10 min. Nonetheless, disinfection on both fingertips and hands was less effective than was suggested by the standard suspension test, this being perhaps the most practically important finding in our study. A similar observation was reported in the above-mentioned study on rhinovirus inactivation by aqueous iodine in vitro and on skin [3]: whereas in vitro 100 μg/ml iodine inactivated rhinovirus within 3 s, a 100-fold iodine concentration plus a 200–400-fold inactivation time were required to eliminate rhinovirus from the skin. However, in that study the volume ratio of disinfectant and virus was not constant. Factors appear to be operating on the skin that protect, in some way or other, the virus from the disinfectant's action.

In the standard suspension test, the influence of biological material in hand disinfection (sheltering of virions in crevices, protein effect) is intended to be imitated by about 3.5% serum included in the reaction mixture. It has not been determined so far whether this is adequate both qualitatively and quantitatively. A higher amount of serum or other material (e.g., yeast) [13] might be required to inhibit virus inactivation to the same degree and by a similar mechanism as skin does.

Another factor that we consider of utmost importance is the evaporation of alcoholic disinfectants in vivo. At least three parameters of disinfection, the significance of which has been shown in vitro and partly in vivo, are altered in the course of evaporation of the disinfectant. One is the volume ratio of disinfectant and virus, shown to be important in the 'Desderman'/poliovirus 1 system (Fig. 2), but probably also significant in other disinfectant virus systems or at other volume ratio ranges. The second parameter is the shortening of the effective inactivation time by complete drying of at least the volatile components of the alcoholic disinfectant. In our experience, the skin of the volunteer felt dry within 30–60 s in the hand test, and 60–120 s in the finger test after application of disinfectant. In routine 'hygienic hand disinfection' recommended for hospital practice, 3 ml disinfectant are applied to both hands and rubbed in for 30 s. Even with the greater disinfectant volume (part of which is usually lost by dropping) the hands feel dry after comparatively short time, 45–120 s. The last parameter influenced by evaporation is the effective temperature on the skin surface. Desirable as it would be to measure the skin surface temperature while disinfectant is evaporating, this was not considered feasible because a thermometer probe in contact with the skin would influence evaporation of

alcoholic disinfectant underneath, and thus temperature. We did not try, however, to measure the infrared radiation of hands without and with disinfectant. The approach we chose was the demonstration of the cooling effect of evaporating ethanol in vitro, and the influence of temperature on virus inactivation in vivo. Taken together the results are evidence that virus disinfection with alcoholic disinfectants may be impaired by a lowering of the effective surface temperature in the course of evaporation.

It should be stressed that, although it appears likely that the parameters discussed above exert an influence in hand disinfection, their relative importance cannot be estimated from the available information. We would, however, tend to assume that evaporation plays a greater role in hand disinfection than the 'sheltering' effects of the skin. Formaldehyde, in contrast to 'Desderman' subject to only little evaporation, was as effective in the in vivo tests as in the suspension test. Furthermore, the 'Desderman' action was less inhibited in the finger than in the hand test, evaporation being slower in the finger test.

We may conclude by mentioning specific advantages and disadvantages of each of the tests for the evaluation of disinfectants. The hand test comes closest to the hand disinfection situation. Volunteers, however, are exposed to large doses of virus which may increase the risk of infection; a number of viruses may not be tested for safety reasons. Furthermore, a large skin surface area is exposed to cell culture medium containing antibiotics, which may lead to skin sensitisation. The specific risks are reduced in the finger test, while essential parameters of in vivo testing are maintained. It appears that compared to hand disinfection 'spontaneous' decay plays a more prominent role, whereas inhibition of disinfection due to disinfectant evaporation or the protein effect of skin may be underrated. Nonetheless, the finger test appears to be a practical model of hand disinfection.

The suspension test in its present form seems to yield too optimistic predictions in the case of at least the naked viruses tested. Its great advantages are its usefulness as a screening test, and its value in elucidating fundamental mechanisms of the disinfectant-virus interaction.

At the present stand of knowledge the evaluation of antiviral hand disinfectants should, in our view, be based on in vivo as well as in vitro testing. The standard suspension test (perhaps with modifications as indicated above), and the finger test are recommended for that purpose.

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