Experiments on Terminal Disinfection by Formaldehyde Vapor in the Case of Smallpox

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The usually recommended terminal disinfection by formaldehyde vapor is unable to completely inactivate vaccinia viruses embedded in scabs. In view of our results, we recommend doubling the concentration of formaldehyde (10 g of formaldehyde per m³ of space) and prolonging the time of exposure to 24 h for terminal disinfection in the case of smallpox. Subsequent disinfection by scrubbing assumes special importance, since no complete inactivation of the scabs occurs.

Disinfection measures to interrupt the chain of infection play a decisive part in the case of smallpox. The living quarters or the patient's room should be terminally disinfected by formaldehyde vapor (1, 4). This is usually done by vaporizing or spraying 5 g of formaldehyde per m³ of space. The mattresses are put in an upright position, and the dressers are opened and the drawers are pulled out. A hygrometer indicates the relative humidity. Finally, the windows and doors are sealed with paper strips. The room to be disinfected must be kept shut for not less than 6 h to allow the formaldehyde to become effective. Subsequent to this the bed and the surrounding area, as well as the furniture, must be thoroughly disinfected by scrubbing. Disinfectants containing formaldehyde are recommended as being particularly suitable for this purpose (1, 3, 4).

When making comparisons of various virus disinfectants it must not be overlooked that there are at present no generally accepted standardized methods for testing virucides. Consequently, the methods used here are based on testing conditions arbitrarily chosen by K. G. (7). Vaccinia virus is usually used as a model for studying the reliability of chemical disinfectants in killing smallpox viruses. This model presents no safety problems for the experimenter and can be said to have approximately the same resistance to disinfectants as the smallpox virus. The remarkable thing is that to date there have been only a few experiments to disinfect pox scabs (2, 5, 10-12). Vaccinia virus more than any other places unusually high demands on the chemical disinfectant used, and scab material must be thoroughly disinfected in the case of smallpox when terminal disinfection by formaldehyde vapor and subsequent disinfection by scrubbing are performed.

Gildemeister et al. (5), who performed extensive experiments on the problem of the efficacy of chemical disinfectants on vaccinia virus as early as 1930, also applied Flügge's procedure to test the reliability of disinfecting rooms by formaldehyde vapor. They discovered that exposing minutely pulverized vaccinia virus scab material to formaldehyde vapor for 4 h did not completely kill the virus. However, it was not absolutely clear whether the reason for this was that the virus embedded in the scab material could not be attacked easily by the formaldehyde or that the full amount of formaldehyde did not evaporate completely. Only 4 g/m³ of space was evaporated instead of 5 g/m³.

To our knowledge, there have as yet been no exhaustive studies made on the effect of treating vaccinia virus scab material with formaldehyde vapor in tests under in vivo conditions, for instance, in sick rooms. This paper, therefore, reports experiments in which scabs containing vaccinia virus (infected rabbit skin) were exposed to terminal disinfection in a sick room. The degree of success of the disinfection can be quantitatively measured by comparing the virus content of the disinfected scabs with the control scab.

MATERIALS AND METHODS

Virus strain. Vaccinia virus strain "Berlin" was used to infect the rabbits; it was obtained from calves. Virus content of 10⁷ to 10⁸ plaque-forming units/ml was tested in 10-day-old chicken embryos by inoculation of the chorioallantoic membrane.

Infection of rabbits. The average weight of the rabbits was 2 to 3 kg. An area of about 8 by 10 cm was scarified and infected with vaccinia virus diluted 1:20 in physiological NaCl solution. About 8 to 10 days later the animals were killed, and the scabs loosely attached to the skin were harvested. The scab material was stored at +4 C.

Preparation of scabs. Shortly before the experi-

ment the scabs (1 to 2 mm thick) were cut into pieces 0.3 to 0.8 cm long and 0.4 to 0.6 cm wide and apportioned for control or experimental purposes. A minimum of eight scabs from two different animals was used for each experiment. Scabs from 14 different rabbits were used in the experiments.

Disinfection procedure. The scab pieces were deposited on glass, cellulose, or linen in an empty tiled room and in a sick room containing two beds and two chairs. A Microsol apparatus built by Hentschke and Sawatzki (model 202F, Neumünster-Gadeland, West Germany) and an Autex apparatus built by Gruss Kommanditgesellschaft (Neuss, West Germany) were used for the terminal disinfection. Both apparatus work as vaporizers. The times of formaldehyde exposure were 6 and 24 h, respectively. Concentration of the formaldehyde was either 5 or 10 g of formaldehyde (equal to 15 to 16 or 30 to 36 ml of formaldehyde solutus DAB 7) per m³ of space. When the disinfection period had expired, the test objects were removed by an assistant, who also read the hygrometer. The formaldehyde could then be neutralized with ammonia.

Isolation of vaccinia virus. The treated, vaccinia virus-containing scabs and the control scabs were minutely cut up, pulverized with sand, and suspended in physiological NaCl solution. The following antibiotics were added: either penicillin and streptomycin or gentamycin and nystatin. A portion of the test material was minutely cut up and used without pulverization. Test systems were: scarified rabbit skin, 10-day-old chicken embryos (inoculation of the chorioallantoic membrane), primary monkey cells, and HeLa cells. (See reference 6 for the technique.) A subculture, using the chorioallantoic membrane, was performed with both rabbit skin and embryonated eggs.

RESULTS

A summary of the results of the terminal disinfection experiments with formaldehyde vapor is given in Table 1. The killing rate was tested by comparing the virus content of the disinfected scabs with that of the control scabs. It can be seen that never more than 85% of the vaccinia viruses in the scabs is killed by the formaldehyde vapor procedure recommended in the smallpox alarm plan (1) (time of exposure, 6 h; 5 g of formaldehyde per m³ of space; more than 75% relative humidity) in the empty rooms as well as in the rooms containing furniture. No significant improvements were observed in the killing rate when the exposure was prolonged. On the other hand, the rate of inactivation of the vaccinia virus reached as much as 97% when the formaldehyde concentration was doubled. An impressive rise in effectiveness was observed in the case of the empty tiled room when the formaldehyde was applied in double concentration, and simultaneously the exposure time was increased from 6 to 24 h. In such cases the rate of inactivation uniformly ex-

ceeded 99.9%. Equally satisfactory effects could not be reached in the case of rooms containing furniture, however.

The scabs treated with formaldehyde vapor were also applied to rabbits after dermal scarification to reveal any false positives in the test systems and to reactivate, if possible, any partially injured virus. No proof of this was obtained in a single case. At intervals, subcultures were set up of the inoculated chorioallantoic membranes and rabbit skin eruptions. The results were always positive.

DISCUSSION

It is not possible to achieve complete inactivation of vaccinia virus scabs (Table 1). These results agree with the findings of Gildemeister et al. (5). The reason for the incomplete inactivation of the virus-containing scabs was probably due, in the case of Gildemeister et al. (5), not to the incomplete evaporation of the formaldehyde but rather to the fact that the viruses were embedded in the scabs. This is not surprising inasmuch as the disinfectant can only penetrate the scab by means of diffusion, which means slowly. The Committee on Formaldehyde Disinfection of the Public Health Laboratory Service also pointed out (2) the difficulties connected with disinfecting smallpox scabs by formaldehyde vapor. Experiments to completely disinfect four scabs from smallpox and alastrim patients, each after 24 h of exposure in special disinfectors, have not been successful.

We were successful in inactivating up to 97% of the vaccinia viruses embedded in scabs by doubling the concentration of formaldehyde. By prolonging the period of exposure from 6 to 24 h, it was possible to enhance considerably the efficacy of the procedure in the case of the empty room, but not in the rooms with furniture. This reveals that the local conditions in each case (for example, the construction of the walls and the furnishings) markedly influence the efficacy of the terminal disinfection. Probably one of the reasons for this is that the relative humidity remains at a high level in an empty room after just one vaporizing treatment with either water or formaldehyde for a longer period than in a furnished room (8). In view of this, we recommend for the time being vaporizing double the amount of formaldehyde and increasing the period of exposure to 24 h for terminal disinfection with formaldehyde in the case of smallpox.

Other authors have already made similar proposals (9) without, however, submitting more detailed descriptions of their testing procedure or corresponding results. In view of the

TABLE 1. Survey of terminal disinfection by formaldehyde vapor^a

Determination	Rooms							
	Empty ^b		Furnished ^c					
	1	2	1		2		3	
			Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Room size (m ³)	51	51	60	60	73	73	73	73
Concentration of formaldehyde/water (ml/ml)	765/765	1,515/1,515	900/900	900/900	1,150/1,750	1,150/1,750	2,400/2,400	2,600/2,600
Room temperature (C)	20-21	20-21	20-22	20-22	20-22	20-22	19-22	19-22
Position of scabs ^d	Glass and cellulose	Glass and cellulose	Glass and linen	Glass and linen	Linen	Linen	Linen	Linen
Time of exposure (h)	6	24	6	24	6	24	24	24
Relative humidity at end of exposure time (%)	93	73-74	80-93	50-65	70-88	50-60	80-95	50-60
Number of experiments ^e	1	3	3	3	3	3	2	2
Virus titer reduction (%)	70	NP, NP, NP	70, 75, 80	80, 75, 70	80, 85, 80	80, 85, 70	90, 95	92, 95
Chicken embryos ^f	80	99.9, 99.9, 99.9	80, 85, 75	72, NP, NP	70, NP, NP	88, NP, NP	95, NP	95, NP
HeLa cells	NP	NP, NP, NP	85, 80, NP	80, NP, NP	NP, NP, NP	NP, NP, NP	95, NP	94, NP
Primary monkey cells								

^a Only one experiment of 6- and 24-h duration was performed with a Microsol apparatus; in all other experiments an Autex apparatus was used (see Materials and Methods). Subcultures were positive in all trials.
^b Rooms were tiled.
^c Furniture included two beds, two chairs, and two side tables.
^d Scabs from 14 rabbits were used in all; for each experiment scabs from two animals were used.
^e Experiments were repeated at approximately 8-day intervals under the same conditions.
^f Ten-day-old chicken embryos were used (chorioallantoic membrane technique). NP, Not performed. Titers given are from one, two, or three trials.

absence of complete inactivation of the virus-containing scabs, the subsequent disinfection by scrubbing after vaporizing formaldehyde assumes great importance. The doctor in attendance and the patient should be made aware of the particular difficulties of disinfecting smallpox scabs. Loose scabs should be removed with tweezers and specially inactivated.

The favorable results obtained with vaporizing formaldehyde in empty rooms should motivate us to carry out further experiments to see to what extent it would be possible to increase the efficacy of terminal disinfection by modifying the procedure.

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