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The effect of relative humidity on swine vesicular disease virus in dried films before and during formaldehyde fumigation

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SUMMARY

Swine Vesicular Disease virus (SVDV) did not survive drying at high relative humidities (r.h.) but there was little virus loss at low r.h.

Purified virus dried in films was inactivated by formaldehyde fumigation only at high r.h. Inactivation was also influenced by the suspending medium from which the virus was dried. Purified virus resuspended in distilled water and then dried, was rapidly killed, but that in tissue culture fluid survived.

INTRODUCTION

Formaldehyde has been used as a disinfectant since the latter part of the nineteenth century. It has powerful batericidal properties and has been used both as a liquid and as a gaseous disinfectant (Walker, 1964). When used as a fumigant, the generation of formaldehyde vapour used to be made from aqueous solutions of formaldehyde (Formalin) by spraying, heating or by exothermic reaction with oxidizing compounds (e.g. potassium dichromate). The P.H.L.S. Committee on Formaldehyde Disinfection (1958) emphasized the necessity for a high humidity for formaldehyde fumigation to be effective. With the advent of the later technique for generating formaldehyde gas from paraformaldehyde, insufficient emphasis appears to be placed on the role of humidity. Taylor, Barbeito & Gremillion (1969), while adjusting the relative humidities to 60% prior to fumigation experiments, suggest that the dry gas liberated from paraformaldehyde may be more effective than vaporized formalin. Beeby, Kingston & Whitehouse (1967) point out that centrally heated buildings in England are likely to have relative humidities as low as 30% and emphasize the necessity for increasing the humidity.

This study was initiated to investigate the effects of humidity, temperature and formaldehyde vapour on dried films containing Swine Vesicular Disease virus (SVDV). Other viruses have been used and will be described in a further publication.

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MATERIALS AND METHODS

Virus

The UGK 27/72 strain of SVDV was used at the third passage level in IB-RS-2 cells (De Castro, 1964) grown in Hanks' medium with lactalbumin hydrolysate (LYH) with 10% tryptose phosphate broth (TPB) and 10% ox serum. The virus suspension was separated from cell debris by centrifugation at 4000 rev./min for 5 min. The supernatant was dispensed as 1 ml samples into Bijou bottles and stored at −20 °C until required.

To detect the influence of residual growth medium upon the survival of virus, purified suspensions of SVDV were prepared by the method used by Brown & Cartwright (1963). 1·0% sodium dodecyl sulphate (SDS) was used to disperse the pellet before sucrose density gradient centrifugation.

Equipment

(1) A sealed glove cabinet (340 l volume) in which some drying and all fumigation experiments were performed was equipped with:

(*a*) An internal fan to maintain constant air circulation.

(*b*) Wet and dry bulb thermometers, used for calculating the relative humidity.

(*c*) A heating mantle containing a metal beaker. Formaldehyde was generated by the introduction of 3·6 g of paraformaldehyde flake. This gave a theoretical yield of 10·6 mg of formaldehyde per litre of air (Hoffman & Spiner, 1970). The elevation of r.h. was achieved by adding water to the heated beaker.

(*d*) A narrow silicone rubber tube (2 mm internal diameter) allowing air samples to be withdrawn for the estimation of formaldehyde by the Nash method (1953).

(*e*) Four glove ports, two of which were fitted with polythene sleeves to allow the passage of samples out of the cabinet.

(*f*) An air extract system capable of removing formaldehyde from the cabinet through a high efficiency filter to atmosphere.

(2) A second glove cabinet operating under negative pressure and with a high efficiency filter on the extract. Bottles containing the samples were introduced and removed through a small port, 5 cm diameter, through which an inward air flow of 100 l/min passed.

(3) Laminar Flow Biological Safety Cabinet (laminar-flow cabinet). This was used for the rapid drying of virus.

Experimental procedure

At the beginning of each experiment a Bijou containing the virus was removed from the −20 °C store, allowed to thaw at room temperature and passed into the laminar-flow cabinet. Samples of 0·05 ml of SVDV suspension, each containing approximately 5×10^7 plaque forming units (p.f.u.), were spread on clean 3×1 cm pieces of glass cut from Chance microscope slides (writing paper cut from Oxford Pad PA4MN was used in one experiment) and dried in the laminar-flow cabinet.

The dried slides were transferred to the first glove cabinet for exposure (see sections 1 and 2 below). At intervals during the experiment a slide was put into a 30 ml wide-mouthed screw-capped bottle (Universal) and inserted into the polythene sleeving for removal. The section of polythene sleeve containing the bottled sample was tied off with adhesive tape, then cut, leaving both the cabinet and the portion of bag containing the sample separated, but sealed from the atmosphere. The sleeved bottle was then introduced to the second cabinet through the port. The sleeve and bottle were opened and the slide was transferred to a Universal containing 5 ml of PBS. This was then removed via the port for virus assay.

Drying-only experiments

The virus films were prepared and exposed to a range of relative humidities and temperatures:

(*a*) Low r.h. was produced in the laminar-flow cabinet, which had a constant air flow and was situated in a laboratory with a low ambient r.h. Production of high r.h. was difficult, therefore only low relative humidity exposures were conducted in the laminar flow cabinet.

(*b*) By vaporizing water, high r.h. was readily produced in the sealed glove cabinet, which had an enclosed volume of air. High relative-humidity drying experiments were therefore conducted in the glove cabinet.

Formaldehyde fumigation experiments

The virus films were dried on slides in the laminar-flow cabinet for 1 h. These were enclosed in a Universal and transferred to the sealed glove cabinet.

Paraformaldehyde flake was then added to the heated beaker. The level of formaldehyde in the cabinet was monitored by withdrawing air samples at intervals and estimating the formaldehyde concentration using the Nash method. It was shown, particularly at high relative humidities, that formaldehyde concentrations were lower than the theoretical yield.

At intervals a slide was placed in an empty Universal. At the same time the wet and dry bulb temperatures were recorded. The bottle was transferred immediately to the second cabinet, the slide was removed from the carrier bottle and placed in a Universal containing PBS.

The purified suspensions were treated in the same manner, but in the second cabinet the slides were introduced into Universals containing 0·1 % SDS in 0·04 M phosphate buffer (to prevent the purified virus from adhering to the surfaces of the container).

Virus assay

Infectivity assays were performed using the plaque technique. IB-RS-2 monolayers were used, and a volume of 0·2 ml of virus dilution was spread onto each of four replicate plates. After incubation at 37 °C for 30 min, 5 ml of overlay medium (LYH + 10 % TPB + 2 % ox serum + 0·7 % (w/v) Noble agar) was added. The plates were then incubated at 37 °C in humidified air with 5 % CO_2, and after 48 h incubation were stained with 10 % formol methylene blue. Plaques were counted and the titre calculated.

Table 1. *Drying*

Temp. (°C)	r.h. (%)	Virus titre $\log_{10}$ p.f.u./ml 0 min	30 min	60 min	90 min	120 min	150 min	180
23	35	6·3	5·95	5·57	5·23	4·85	4·50	4·15
24	54	6·1	5·45	4·85	4·23	4·60	3·00	—
24	86	6·2	3·05	3·95	2·88	1·80	0·70	0·0
33	76	6·7	3·75	0·95	0·0	0·0	0·0	0·0

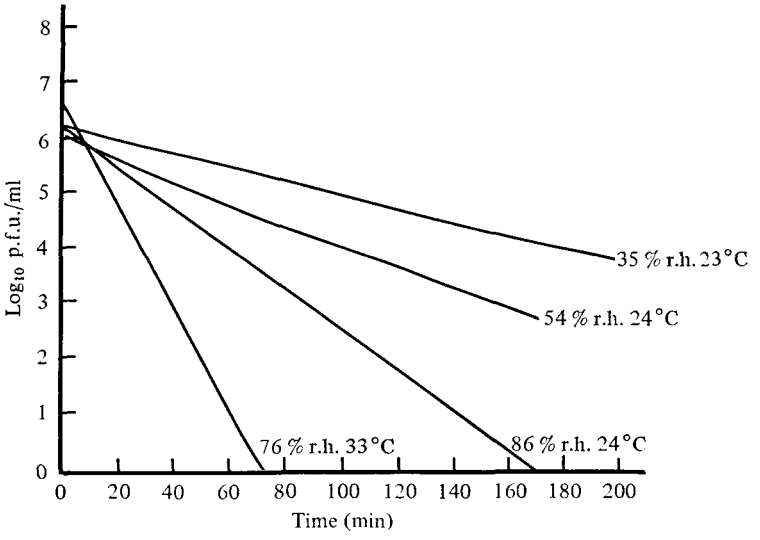

Fig. 1. Drying.

Table 2. *Exposure of dry films to raised relative humidity and temperature*

Temp. (°C)	r.h. (%)	Virus titre $\log_{10}$ p.f.u./ml 0 min	30 min	60 min	90 min	120 min	150 min	180 min
34	76	4·30	0·65	0·0	0·0	0·0	0·0	0·0

RESULTS

Films of SVDV were dried on glass slides at various temperatures and r.h. The maximum survival of virus was achieved when the r.h. was low and therefore the drying was rapid (Table 1; Fig. 1). SVDV dried slowly in a high relative humidity atmosphere was destroyed within 3 h. A period of 1 h at a low r.h. was chosen as a suitable drying time. At the end of this drying period the virus film appeared to be dry and the experimental data indicate that the rate of decrease in virus titre was low. Rapidly dried virus subsequently exposed to a high relative humidity atmosphere did not survive (Table 2; Fig. 2). When virus was dried rapidly to obtain maximum survival and then exposed to a high relative-humidity atmosphere containing formaldehyde (Table 3; Fig. 3) little virus loss was observed.

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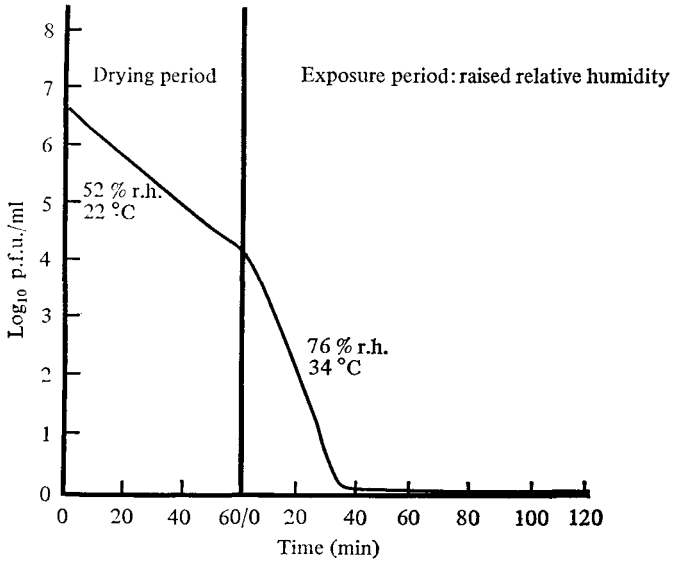

Fig. 2. Exposure of dry films to raised relative humidity and temperature.

Table 3. *Fumigation of dried virus films*

Temp. (°C)	r.h. (%)	CHOH (mg/l)	Virus titre $\log_{10}$ p.f.u./ml 0 min	20 min	40 min	60 min	80 min	100 min	120 min
25	56	8·18	5·5	5·00	4·85	4·70	4·55	4·43	4·30
34	68	4·65	5·1	4·20	4·18	4·15	4·15	4·15	4·13
35*	71	2·60	3·4	3·35	3·28	3·23	3·18	3·13	3·08
35	61	4·40	5·1	5·00	4·90	4·80	4·65	4·55	4·45

* Paper used instead of glass slides.

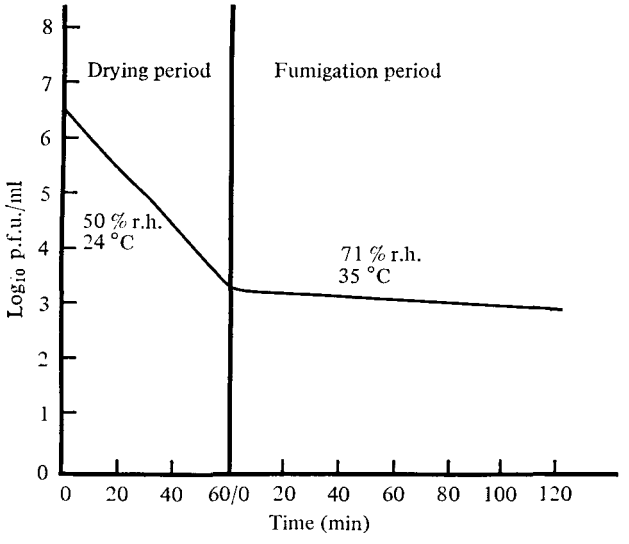

Fig. 3. Fumigation of dried virus films.

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Table 4. *Fumigation of purified SVDV*

Temp. (°C)	r.h. (%)	CHOH (mg/l)	Virus titre $\log_{10}$ p.f.u./ml 0 min	20 min	40 min	60 min	80 min	100 min	120 min
28*	53	5·20	3·7	0·0	0·0	0·0	0·0	0·0	0·0
32*	79	8·56	5·2	0·0	0·0	0·0	0·0	0·0	0·0
28†	53	8·00	5·6	3·4	2·1	1·7	0·6	0·0	0·0

* Suspended in distilled water.
† Suspended in LYH + 10% TPB + 10% ox serum.

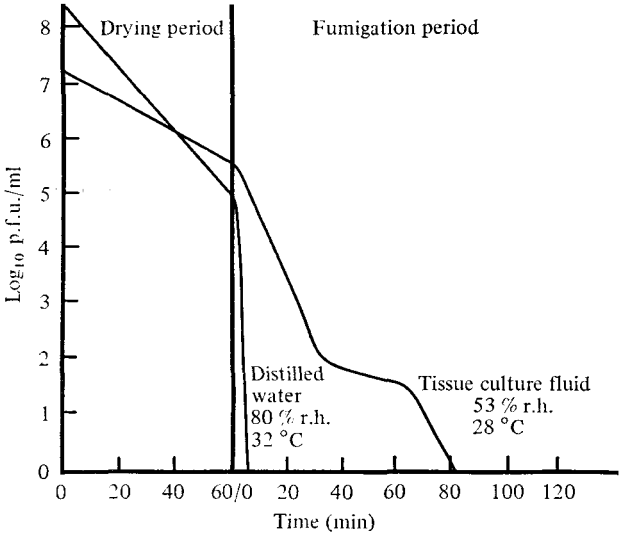

Fig. 4. Fumigation of purified SVDV.

At 71% r.h. and 35 °C, only $10^{0·3}$ p.f.u./ml SVDV were lost in 2 h. This would seem to suggest that formaldehyde gives a certain amount of protection to the virus, which would otherwise have been destroyed because of the high r.h.

The preparation of purified SVDV suspended in distilled water and then dried on the glass slides was destroyed by formaldehyde (Table 4; Fig. 4). Purified SVDV suspended in tissue culture fluid and dried as a film seemed to be protected as were the non-purified preparations.

DISCUSSION

In formaldehyde inactivation of virus vaccines, problems were observed which suggested that a first-order inactivation was not occurring; Salk *et al.* (1954) observed this with poliovirus. The formation of aggregates and precipitates were presumed to shield small quantities of the virus from the action of the formaldehyde. Filtration to remove this material was recommended. A similar situation indicating survival of foot-and-mouth disease virus has been observed by Wesslén & Dinter (1957) in vaccines inactivated by formalin. Bachrach *et al.* (1957) were

unable to define the complete inactivation of FMDV though the early inactivation was in accordance with first-order kinetics.

The tanning and protein fixation effects of formaldehyde are well known. In considering the action of formaldehyde on the aggregates in virus vaccine preparation, Gard & Maaløe (1959) suggest that formaldehyde might be expected to penetrate the protein coat by diffusion and that a progressive fixation or tanning of the protein will lower its permeability. Thus a membrane effect is to be expected, manifesting itself in a gradual decrease in the rate of inactivation.

This phenomenon appears to be the factor influencing the survival at low r.h. It is considered that a highly effective barrier of fixed protein is rapidly established at the surface; this not only blocks the deeper penetration of formaldehyde but seems to impede water penetration from high r.h. atmospheres subsequent to formaldehyde exposure. This work emphasizes the importance of high r.h. in fumigation and that the optimum results are obtained by exposing the objects for fumigation to a conditioning period of high r.h. before fumigation. These suggestions pertain especially to the modern air-conditioned laboratory where operating r.h. is usually low.

REFERENCES

Bachrach, H. L., Breese, S. S., Callis, J. J., Hess, W. R. & Patty, R. E. (1957). Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. *Proceedings of the Society for Experimental Biology and Medicine* **95**, 147.

Beeby, M. M., Kingston, D. & Whitehouse, C. E. (1967). Experiments on terminal disinfection of cubicles with formaldehyde. *Journal of Hygiene* **65**, 115.

Brown, F. & Cartwright, B. (1963). Purification of radioactive foot-and-mouth disease virus. *Nature, London* **199**, 1168.

De Castro, M. P. (1964). Behaviour of the foot-and-mouth disease virus in cell cultures: suceptibility of the IB-RS-2 cell line. *Archivos do Instituto biologico, São Paulo* **31**, 63.

Gard, S. & Maaløe, O. (1959). Inactivation of viruses. In *The Viruses* vol. 1, (ed. F. M. Burnett and W. D. Stanley), ch. v. New York and London: Academic Press.

Hoffman, K. & Spiner, D. (1970). Effect of relative humidity on the pentrability and sporicidal activity of formaldehyde. *Applied Microbiology* **20**, 611.

Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch Reaction. *Biochemical Journal* **55**, 416.

P.H.L.S.Committee on Formaldehyde Disinfection (1958). The practical aspects of formaldehyde fumigation. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **17**, 270.

Salk, J. E., Krech, U., Youngner, J. S., Bennett, M. B., Lewis, L. J. & Bazeley, P. L. (1954). Formaldehyde treatment and safety testing of experimental poliomyelitis vaccines. *American Journal of Public Health* **44**, 563.

Taylor, L. A., Barbeito, M. S. & Gremillion, G. G. (1969). Paraformaldehyde for surface sterilization and detoxification. *Applied Microbiology* **17**, 614.

Walker, J. F. *Formaldehyde*, 3rd ed. (1964), p. 570. New York: Reinhold.

Wesslén, T. & Dinter, Z. (1957). The inactivation of foot-and-mouth disease virus by formalin. *Archiv für die gesamte Virusforschung* **7**, 394.