The Influence of Various Chemical Additives on the Survival of Vesicular Exanthema Virus in Aerosols

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SUMMARY

The influence of relative humidity (r.h.) on the survival of vesicular exanthema virus (VEV) in aerosols at 1 s and during the next 5 min when generated from phosphate buffer solution containing polyhydroxy compounds, dimethyl sulphoxide, salt or protein has been examined. VEV was sensitive to r.h. in the range of 40 to 60% in the presence of bovine serum albumin, glucose, inositol or phosphate buffer. Addition of sodium chloride stabilized the virus in aerosols at mid-range r.h. both immediately after generation and after a period of storage for 5 min. In the presence of dimethyl sulphoxide or glycerol, virus survival was reduced at 20% r.h. at 1 s and at all r.h. during the first 5 min. Pre-humidification did not produce any significant difference in virus recovery.

It has previously been found that two caliciviruses, vesicular exanthema virus (VEV) and feline calicivirus, are most stable in aerosols at low relative humidity (r.h. 20 to 30%) and high r.h. (70 to 80%) and least stable at intermediate r.h. values (40 to 60%; Donaldson & Ferris, 1976). The r.h. survival pattern of these viruses therefore differs from that of other very small naked animal viruses where good survival at high r.h. but poor survival at low r.h. has been observed, with 50 to 60% r.h. being the critical region separating good from poor survival (Hemmes et al. 1960; Akers et al. 1966; Benbough, 1971; Barlow, 1972a; Donaldson, 1972; de Jong et al. 1974; Donaldson & Ferris, 1974, 1976). These differences might possibly be due to the composition of the suspending fluid from which the aerosols were generated, as this can markedly affect virus survival in aerosols in relation to r.h. (Harper 1961, 1963; Webb et al. 1963; Benbough, 1969, 1971; Barlow, 1972b; Schaffler et al. 1976).

In this present study, virus suspensions of VEV were prepared, in which host protein and salt components were removed. The effect of chemical additives such as polyhydroxy compounds, dimethyl sulphoxide, protein and salt on the stability of VEV in aerosols was then investigated to determine if mid-range sensitivity was maintained.

With some viruses, pre-humidification before aerosol collection has been shown to increase the amount of infectivity recovered (Hatch & Warren, 1969; Warren et al. 1969; Dubovi & Akers, 1970; Benbough, 1971; Barlow, 1972b; de Jong et al. 1975), indicating that inactivation of these viruses in aerosols occurs during rehydration. The effect of pre-humidification on the recovery of VEV infectivity in aerosols has also been examined.

Vesicular exanthema virus (VEV, type E) was obtained from Mr J. N. Burroughs of this Institute and grown in monolayers of IB-RS-2 cells (de Castro, 1964) contained in 20 oz medical bottles, using Eagle's medium for maintenance. When cells showed maximal c.p.e., usually at 8 h after infection, the bottles were shaken to release any remaining cells adhering to the glass into the suspending fluid. The medium from several bottles was pooled and the cells and debris were pelleted by centrifugation at 3000 rev/min for 15 min, resuspended in 5 ml of 0.01 M-phosphate-buffered saline (PBS), pH 7.6, freeze-thawed and sonicated to
release virus (Zee et al. 1968). The suspension was then clarified by centrifugation at 10,000 rev/min for 30 min.

Host protein material was removed by applying 3 ml of concentrated virus suspension to a 30 x 1.5 cm column of Sepharose 4B (Pharmacia, 75 Uxbridge Road, London W5 5SS) and eluting with 0.04 M-PBS, pH 7.2. The peak virus fractions were identified by plaque assay in IB-RS-2 cells and then pooled, subdivided and stored in 5 ml aliquots at -70 °C. Salt was initially removed from concentrated virus suspensions by applying 6 ml of de-proteinized material to a 40 x 3 cm column of Sephadex G-25 (Pharmacia) and eluting with glass-distilled water, pH 7.0. However, a 6 log reduction in virus infectivity occurred, producing a virus preparation of insufficient infectivity for experimental purposes. However, virus was stable in 0.01 M-phosphate buffer and this was subsequently used as the basic suspending medium.

Plaque assays were carried out using IB-RS-2 cells grown in 6 cm Petri dishes and from three to five dishes were used for each virus dilution. The cells were overlaid with Earle's yeast lactalbumin hydrolysate medium containing 2.5% ox serum and 1% Noble agar (Wawrzkiewicz et al. 1968) and stained with neutral red after incubation at 37 °C for 4 days in air containing 5% CO₂.

A detailed description of the aerosol test procedures used and the calculation of results has been given previously (Donaldson, 1972). Aerosols were generated using a Collison spray with three spray heads (May, 1973). The aerosols were held within a portable Henderson apparatus (Druett, 1969) with attached 75 l rotating drum (Goldberg et al. 1958) containing air at controlled r.h. The drum was rotated at 4 rev/min to minimize physical loss of aerosol cloud by sedimentation. Samples of the cloud were collected with Porton raised all-glass impingers (May & Harper, 1957) containing 10 ml of PBS with 0.25% (w/v) bovine serum albumin (BSA) and 0.1% silicone antifoam emulsion. Aerosol samples were collected 1 s after generation and after 5 min storage in the drum. The experiments were performed at the ambient temperature (18 to 23 °C) and from three to five tests were made at each r.h., the r.h. being determined by wet-dry bulb psychrometry.

Equal volumes of concentrated, de-proteinized and desalted virus suspensions in 0.01 M-phosphate buffer were mixed with either a 10% (w/v or v/v) buffer solution of a polyhydroxy compound (glucose, inositol, glycerol), dimethyl sulphoxide (DMSO) or NaCl to give a 5% (w/v or v/v) solution. Virus suspensions were also mixed with BSA to give a 0.1% (w/v) solution. The pH of the suspensions was between 7.2 and 7.4. The components were added to the virus suspension immediately before commencement of aerosol generation.

The influence of humidification on the recoveries of infectivity of virus in aerosols generated from phosphate buffer at 1 s was determined. A flask containing a small volume of water was heated to ensure that the r.h. in the vessel was 100%. The heat source was removed and virus aerosols were drawn through this vessel, maintained at 100% r.h. prior to collection.

The log percentage viabilities of VEV in aerosols at 1 s and 5 min with respect to r.h. are shown in Fig. 1. From Fig. 1(a) it can be seen that VEV in aerosols generated from glucose, phosphate buffer, BSA, DMSO and inositol suspensions showed sensitivity at 1 s to r.h. in the 40 to 60% range. At this mid-range r.h. there was least survival of virus in the presence of inositol. The addition of NaCl to de-proteinized and desalted virus preparations increased the survival of infective virus in aerosols during the first 1 s at 60% r.h., while addition of glycerol was particularly unfavourable for survival at low r.h.

The basic pattern of mid-range sensitivity of virus in aerosols was maintained when aerosols were produced from phosphate buffer, BSA, glucose and inositol preparations and recoveries determined after a 5 min storage period (Fig. 1b). As with 1 s survivals, virus was
Fig. 1. Survival of vesicular exanthema virus in aerosols sampled (a) 1 s and (b) 5 min after generation from: ○—○, 0.01 M-phosphate buffer; ●—●, 5% glycerol; •—•, 5% sodium chloride; □—□, 5% inositol; Δ—Δ, 5% glucose; ▲—▲, 5% DMSO; ■—■, 0.1% BSA.

protected against inactivation at mid-range r.h. during the next 5 min in the presence of NaCl. The influence of DMSO and glycerol on VEV survival was markedly different from that obtained with the other five additives. Viability during the first 5 min was considerably reduced in the presence of DMSO (recoveries being the lowest at 40, 60 and 80% in comparison with amounts obtained with other additives at these r.h.), even though the general pattern of mid-range sensitivity was maintained. Glycerol was particularly unfavourable for virus survival in aerosols at 20 and 40% r.h.

Percentage recoveries of VEV in aerosols at 1 s with pre-humidification at 20, 40, 60 and 80% r.h. were not significantly different from those obtained without pre-humidification.

In the presence of glucose, phosphate buffer, BSA or inositol, virus in aerosols was sensitive to r.h. in the range 40 to 60%. The pattern of recovery of virus infectivity in aerosols was therefore similar to that obtained in a previous study (Donaldson & Ferris, 1976). The addition of DMSO and glycerol produced additional inactivation of infectivity during a 5 min period after generation at 20, 40 and 60% r.h. This feature is in contrast to that found for foot-and-mouth disease virus (FMDV), although inactivation occurred during the freeze-drying of this virus in the presence of these chemicals (Barlow, 1972b). The reduction in recovery may be due to toxic concentrations of the chemicals forming during
the process of dehydration (Benbough, 1971; Trouwborst & de Jong, 1973). DMSO can replace water molecules as hydrogen bond acceptors and induce conformational changes in the nucleic acid by disrupting the hydrogen-bond cross-linking between purine and pyrimidine bases (Szmant, 1971).

NaCl increased the stability of VEV in aerosols at mid-range r.h.; this is a similar effect to that which Benbough (1971) obtained with poliovirus at high r.h. He suggested that inactivation of non-lipid viruses occurred during rehydration and the enhancement in virus recovery by NaCl may have resulted from a modification of the rehydration rate during collection. However, pre-humidification failed to reduce inactivation of VEV, which is in contrast to the findings for FMDV (Barlow, 1972b) and poliovirus (Benbough, 1971) but similar to results obtained with mengovirus (Warren et al. 1969).

Inositol failed to protect against virus inactivation at unfavourable r.h.; again, this differs from Barlow's results (1972b) with FMDV, where inositol enhanced viabilities below 45% r.h. The failure of inositol to protect against virus inactivation is surprising, as it has been suggested to be able to replace water lost during dehydration and, hence, to maintain the biological integrity of the virus particle (Webb et al. 1963; Webb, 1965).

Until recently, caliciviruses were included in the Picornaviridae family (Fenner, 1976) but it is now considered that they are sufficiently different in morphology, structure and replication from picornaviruses to be included in a new family, the Caliciviridae (Andrewes et al. 1978). The survival profile of caliciviruses in aerosols is therefore markedly different from that of other very small naked animal viruses and the reasons for this may lie in differences in structure. These results lend further support to the proposed re-classification of caliciviruses.

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REFERENCES


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