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Some Factors Affecting the Survival of Airborne Viruses

By J. E. BENBOUGH
Microbiological Research Establishment, Porton, Nr Salisbury, Wiltshire

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SUMMARY

The response of aerosolized viruses to relative humidity depended greatly on the composition of the fluid from which the viruses were sprayed. For example, the removal of salts from the spray fluid diminished the loss of infectivity of Langat virus (a group B arbovirus) at intermediate relative humidities. Salts were less toxic towards aerosolized Semliki Forest virus (a group A arbovirus) but did cause some loss of infectivity at higher relative humidities after prolonged storage of the aerosol. Polyhydroxy-compounds reversed the virucidal effects of salts on arboviruses. The removal of protein from the spray suspensions of arboviruses caused rapid loss of infectivity in the aerosol at very high relative humidities but had no detrimental effect at lower relative humidities. Poliovirus and T coliphage, which possess no structural lipid, retained high levels of infectivity following aerosolization at relative humidites of 70 % or above; at lower relative humidities they were inactivated rapidly. This rapid inactivation was increased further at lower solute concentrations of the spray fluid.

The infectivities of aerosolized polioviruses and coliphages depended on the mode of rehydration during collection of the aerosols, but the infectivities of arboviruses in aerosols were unaffected by this. Atmospheric oxygen was not toxic to viruses in the aerosol state.

INTRODUCTION

Some information has already been obtained on the effect of relative humidity on the retention of virus infectivity in stored aerosols (Harper, 1961, 1963*a*, *b*; Ehrlich, Miller & Idoine, 1964; Benbough, 1969). Aerosolized influenza virus was shown to be inactivated rapidly at high relative humidities but not at low relative humidities (Hemmes, Winkler & Kool, 1960). The same was shown for measles virus, except that inactivation was more rapid at relative humidities of 60 to 80 % than at higher relative humidities (De Jong & Winkler, 1964). Aerosolized poliovirus retained its infectivity for a long time at relative humidities of 70 % or above, but its inactivation increased with decreased relative humidities below 70 % (De Jong & Winkler, 1968). These authors concluded that viruses with structural lipids survived best in aerosols at low relative humidity, while ether-resistant viruses without structural lipids generally survived best at high humidities. Sanger (1967) examined the inactivation rates of many aerosolized viruses as a function of relative humidity and was unable to confirm this generalization. In all of these studies the suspending fluids were not well defined, and it may be that this confused the relationship between structure and survival of infectivity, as suggested by the studies of Harper (1963*a*) and Benbough (1969). In this work an attempt was made to remove defined components of the suspending medium

and to measure their effect upon the survival of virus infectivity. These procedures have been applied to Semliki Forest virus, Langat virus, poliovirus and the T1, T2 and T7 coliphages.

METHODS

Growth of viruses. Semliki Forest virus (derived from the original strain VR67 of the American Type Culture Collection) was grown in stirred suspensions of chick embryo cells by the method of Zwartouw & Algar (1968). The Langat virus inoculum was the 14th mouse passage of the original Tick Pool 21 strain, and the virus was grown in the same way as Semliki Forest virus, except that actinomycin D (Merck, Sharp & Dohme, Research Laboratories, Rahway, New York) was included in the suspension (H. T. Zwartouw & G. A. Phillips, personal communication). In both cases the clarified culture fluids contained approximately 10^9 p.f.u. in medium 199 with 10 % calf serum.

The standard Sabin attenuated type 1 (LSc2ab) strain of poliovirus was used. Thirty ml. of a suspension of HeLa cells containing 1×10^7 cells/ml. in medium 199 were put into a 1000 ml. Roux bottle. To this were added 25 ml medium 199 containing 10 % calf serum and 0·1 % (w/v) additional sodium bicarbonate. The mixture was inoculated with 1·0 ml. of the virus seed containing 10^8 p.f.u. (Virus Reference Laboratory, Colindale Avenue, London, N.W. 9) and then incubated at 37° for 24 hr in an atmosphere of 5 % carbon dioxide in air. The cell debris was removed by centrifugation. The clarified culture fluid contained approximately 5×10^8 p.f.u./ml.

T coliphages were produced from *Eschericha coli* B growing exponentially in liver digest broth (1·5 % (w/v) Oxoid protease peptone, 0·25 % (w/v) Oxoid liver digest, 0·5 % (w/v) Oxoid yeast extract and 0·5 % (w/v) NaCl in water brought to pH 7·4 with NaOH solution). This was done by transferring 1·0 ml. of a suspension of exponentially growing *E. coli* B, containing approximately 4×10^9 organisms/ml., to 20 ml. of aerated fresh liver digest broth at 37° to which 1·0 ml. of the stock coliphage suspension containing 5×10^9 p.f.u. was added. Complete lysis occurred after incubation for 30 min. The cell debris was removed by centrifugation and the clarified culture fluid contained about 5×10^{10} p.f.u./ml.

Plaque assay of viruses. Semliki Forest and Langat viruses were assayed by the method of Bradish & Allner (1967) in which plaques are produced in suspensions of chick embryo cells in agar. For the assay of Langat virus the chick embryo cells were washed twice in medium 199 before use. Five ml. of 1·5 % (w/v) Oxoid Ionagar No. 2 in medium 199 containing 25 mg./ml. actinomycin D were thoroughly mixed with 1·0 ml. of a dilution of the virus sample and 4·0 ml. of chick embryo cells ($5·0 \times 10^7$ cells/ml.) suspended in medium 199 containing 10 % calf serum and 25,000 units/ml. Colomycin (Pharmax Ltd, Crayford, Kent). This molten mixture was maintained at 45° for the short period before being poured into 9 cm. disposable Petri dishes. After incubation for 5 days at 34° in an atmosphere of 5 % carbon dioxide in air the cultures were stained with 0·05 % (w/v) neutral red solution and incubated for a further 2 hr. The virus sample was assayed at a number of serial threefold dilutions and that yielding 50 to 60 plaques/dish was counted. Six such dishes yielded plaque counts within a range of up to ± 10 %.

For the assay of poliovirus, 1·0 ml. of diluted virus suspension was added to 4·0 ml. of HeLa cells (1×10^7/ml.) suspended in medium 199 containing 10 % calf serum. This was mixed thoroughly with 5·0 ml. of 1·5 % (w/v) Oxoid Noble agar in medium 199 containing 0·2 % (w/v) additional sodium bicarbonate: this molten mixture was kept at 45° until poured into Petri dishes. The plates were incubated at 37° for 3 days in an atmosphere of 5 % carbon dioxide in air. The cultures were stained with 0·15 % (w/v) 2-(*p*-iodophenyl)-3-*p*-nitrophenyl-

5-phenyl tetrazolium chloride (B.D.H. Ltd, Poole) in 0·9 % (w/v) saline and incubated for a further 4 hr.

T coliphages were assayed by the formation of plaques on lawns of *E. coli* B on blood agar base (1·5 % (w/v) Oxoid protease peptone, 0·25 % (w/v) Oxoid liver digest, 0·5 % (w/v) Oxoid yeast extract, 0·5 % (w/v) NaCl, 0·5 % (w/v) Oxoid agar in water brought to pH 74 with NaOH). Five ml. of molten blood agar base containing a drop of an exponentially growing culture of *E. coli* B (approximately 10^{10} organisms/ml.) were thoroughly mixed with 1·0 ml. of diluted coliphage suspension before being poured on to the surface of solidified nutrient agar plates. Plaques were counted following incubation at 37° for 16 hr.

Removal of salts and host protein from clarified virus suspension. Host protein material was adsorbed on to columns of Sepharose 4B (Pharmacia, Uppsala, Sweden) and the virus was eluted with phosphate-buffered saline. Salt ions and other small molecular species were removed from the virus suspensions using columns of Sephadex C-25 (Pharmacia, Uppsala, Sweden) and the virus was eluted with distilled water (Benbough, 1969). Such eluates contained virus in a medium comprising less than 0·5 μg./ml. Cl^- ions and 1 μg./ml. PO_4^- ions and 2 μg./ml. of protein.

Aerosol generation, storage and collection. Aerosols containing virus and tracer were generated by a 3-jet Collison Spray. The spray fluid of 10 ml. total volume contained about 10^9 p.f.u./ml. The characteristics of the Collison spray are such that this fluid yields essentially monodisperse aerosol (Green & Lane, 1957). The cloud was stored in a 120 l. rotating drum containing air at a controlled relative humidity. The aerosols were collected by Porton-raised impingers (May & Harper, 1957) containing collecting fluid incorporating a small quantity of antifoam agent. Phosphate-buffered saline at pH 7·4 containing 10 % calf serum was the collecting fluid for aerosols of arboviruses and poliovirus; the blood base broth was used for collecting coliphage aerosols. Bacterial aerosols are usually traced with washed spores or killed radio-labelled bacteria (Cox, 1966; Harper, 1961). Such methods might not be ideal for tracing viruses in aerosols sprayed from fluids of low solute content because of the difference in size between particles containing virus and those containing tracer: for this reason T7 coliphage containing ^{32}P-labelled DNA was tried as a tracer in this case. The suspension containing radio-labelled T7 coliphage was clarified by centrifugation, desalted and deproteinized before being added as tracer to the test virus suspension. In later experiments labelled phosphate of high specific activity was used as an alternative tracer of viruses sprayed from fluids of low solute content and the results obtained were identical to those with the purified radio-labelled T coliphage as tracer. Radioactive sodium phosphate (specific activity 44 c/μg. P) at concentrations not exceeding 0·005 μg./ml. of spray fluid was therefore used as the routine tracer. The radioactivity in 0·5 ml. quantities of diluted spray fluids and impinger samples was measured after mixing with 9·5 ml. of scintillation fluid, using a Phillips Automatic Liquid Scintillation Analyser. The scintillation fluid was prepared just before use and consisted of N.E. 220, dioxane and formamide in the ratio of 10:8:1; all were scintillation grade compounds obtained from Nuclear Enterprises (G.B.) Ltd, Edinburgh.

RESULTS

Measurements of virus infectivity in the spray fluid immediately before and after each spraying experiment showed that mechanical inactivation of viruses during the 2 min. operation was negligible.

Survival of Langat virus infectivity in aerosols

When Langat virus was sprayed from clarified culture fluid the survival of infectivity was sensitive to relative humidity (Fig. 1). At low relative humidities (around 20 %) there was a rapid initial inactivation of virus within 1 sec. of spraying followed by a very low inactivation rate; at high relative humidities (70 % or over) there was a smaller initial inactivation followed by continuous inactivation during the storage period. A very rapid inactivation of the infectivity of Langat virus occurred in aerosols within the relative humidity range from 40 to 75 %. Protection against this loss of infectivity at intermediate relative humidities was provided when 5 % of a polyhydroxy-compound (glucose, inositol, sorbitol) was added to the spray fluid (Fig. 2).

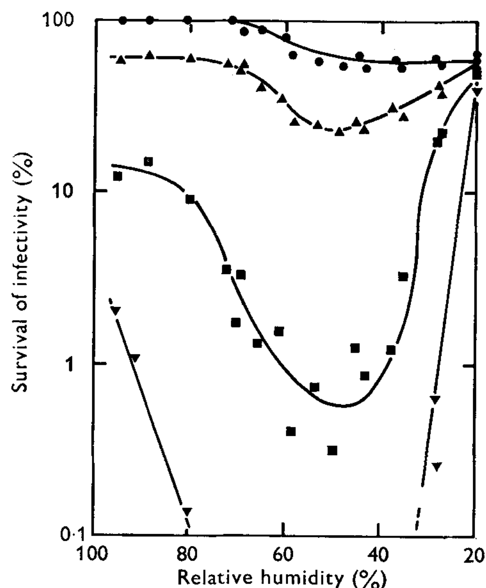

Fig. 1

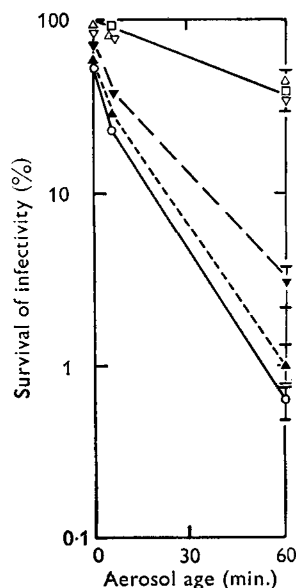

Fig. 2

Fig. 1. The influence of relative humidity on the survival of Langat virus in aerosols sprayed from culture fluid. Aerosol age: ●, 1 sec.; ▲, 5 min.; ■, 1 hr.; ▼, 3 hr.

Fig. 2. The survival of aerosolized Langat virus at 50 % relative humidity when sprayed from culture fluid containing the following quantities of polyhydroxy-compounds. ○, none; ▲, 0·05 % (w/v) inositol; ▼, 0·5 % (w/v) inositol; □, 5·0 % (w/v) inositol; △, 5·0 % (w/v) sorbitol; ▽, 5·0 % (w/v) glucose.

Fig. 1 and 3 show a marked change in the survival of the infectivity of Langat virus and in its dependence on relative humidity when salts were removed from the clarified culture fluid before spraying. The greatest difference occurred at intermediate relative humidities when the recovery of infectivity from aerosols after 1 hr was improved approximately 50-fold. The addition of salts to previously desalted culture fluids produced a significant change in the influence of relative humidity on virus survival in aerosols. When sprayed from 5 % (w/v) solutions of NaCl or KCl, virus infectivity was rapidly inactivated at intermediate relative humidities but not at higher or lower relative humidities: the rates of inactivation were greater for NaCl than for KCl. When virus was sprayed from a 5 % (w/v) solution of LiCl the inactivation rate was not affected at very high relative humidities. At

lower relative humidities the inactivation rate increased, so that below 50 % no infective virus could be recovered in aerosols within 1 min. after spraying (Fig. 4). The survival of infectivity for Langat virus in aerosols depended on the concentration of NaCl in the spray fluid (Table 1).

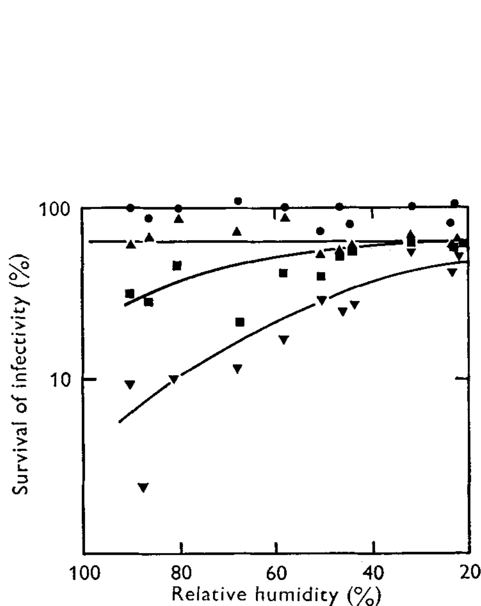

Fig. 3

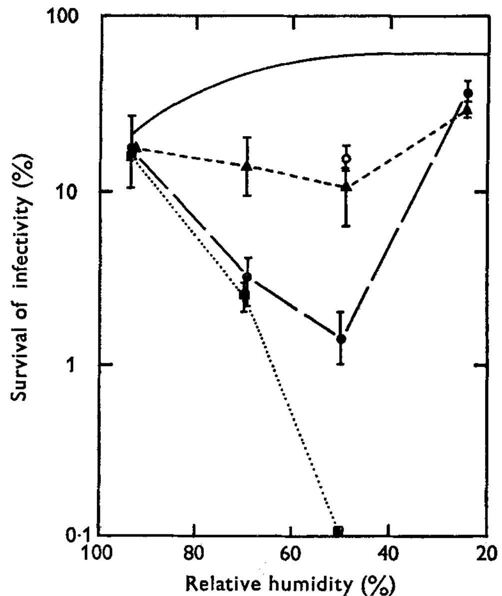

Fig. 4

Fig. 3. The survival of aerosolized Langat virus sprayed from desalted suspension as a function of the relative humidity at the following aerosol ages. ●, 1 sec.; ▲, 5 min.; ■ 1 hr.; ▼, 3 hr.

Fig. 4. The effect of added salts to a previously desalted spray suspension on the survival of aerosolized Langat virus after 1 hr as a function of relative humidity. —, No added salts (as Fig. 3); ○, 0·1 % (w/v) NaCl; ●, 5·0 % (w/v) NaCl; ▲, 5·0 % (w/v) KCl; ■, 5·0 % (w/v) LiCl.

Table 1. *The effect of sodium chloride concentration in the spray fluid on the infectivity of Langat virus aerosols at* 50 % *relative humidity*

Concentration of NaCl in spray fluid, g./l.	Aerosol age		
	1 sec.	5 min.	1 hr
	Recovery of infectivity (%)		
0·00	90 ± 5	62 ± 6	51 ± 6
0·01	95 ± 5	65 ± 5	19 ± 8
0·05	91 ± 5	30 ± 7	10 ± 7
0·50	88 ± 4	9 ± 3	2 ± 1

When the concentration of protein in suspensions of Langat virus was lowered by Sepharose treatment, this had little effect on the recovery of infectivity from aerosols at relative humidities below 80 %, but it increased the rate of inactivation in aerosols stored at high relative humidities (Fig. 5). At 90 % relative humidity the infectivity of the aerosolized virus diminished by 100-fold within 1 min. of spraying, and this loss was prevented by the addition to the spray fluid of 0·1 % (w/v) bovine serum albumin. Thus the infectivity of Langat virus was inactivated rapidly by spraying from suspensions of low protein content, as shown for Semliki Forest virus (Benbough, 1969).

Survival of Semliki Forest virus infectivity in aerosols

The survival of infectivity of Semliki Forest virus in aerosols sprayed from various suspending fluids has been described (Benbough, 1969). Langat and Semliki Forest viruses survive similarly in aerosols sprayed from solute-free suspensions (Fig. 6) but Langat virus is much more sensitive to further inactivation by salts.

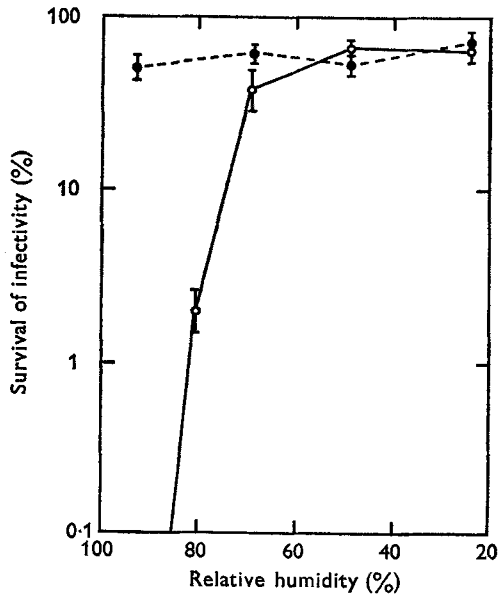

Fig. 5

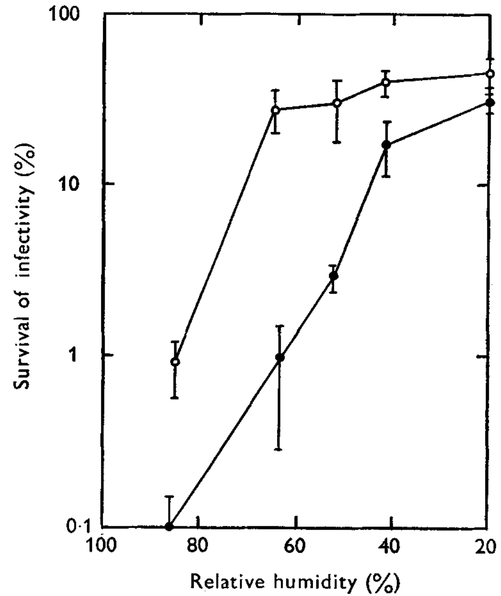

Fig. 6

Fig. 5. The survival of aerosolized Langat virus after 1 hr. as a function of the relative humidity. ○, Sprayed as desalted and deproteinized suspension; ●, sprayed as desalted and deproteinized suspension to which bovine serum albumin (final concentration 0·1 %, w/v) was added.

Fig. 6. The survival of Semliki Forest virus after 24 hr. as a function of the relative humidity when originally sprayed from: ○, desalted suspension; ●, desalted suspension with 5 % (w/v) NaCl.

The survival of T coliphages in aerosols

T2 and T7 coliphages sprayed from the supernatant fluids of the centrifuged bacterial lysates displayed survival patterns similar to those for T3 (Ehrlich *et al.* 1964). The survival of T7 coliphage (Fig. 7) depended on the relative humidity at which the aerosol was held. At relative humidities above 70 % the coliphage showed about 70 to 100 % survival after storage for 1 hr, but at relative humidities below 70 % infectivity decreased rapidly. Thus, at 40 % relative humidity only 0·1 % of virus was infective at 10 min. after spraying, whereas at 72 % relative humidity, 10 % was infective at 24 hr after spraying. Similar tests using T2 phage showed that it was more stable in the aerosol than was T7 coliphage, although the survival pattern was similar. The addition of polydroxy-compounds to the spray fluid did not enhance the survival of these coliphages even at low relative humidities. If salts and proteins were removed from the suspending fluid for T2 and T7 coliphages before spraying, only 0·1 to 1 % of infectivity was recovered in aerosols at relative humidities below 90 % (Fig. 8). The addition of salt or glucose, or of many other soluble substances, to such

suspensions of very low salt and protein content changed the decay pattern so that the rapid loss of coliphage infectivity within 1 sec. of spraying was not greatly reduced but the subsequent decay rate was considerably slower (Fig. 9).

When aerosols of T2 and T7 coliphage previously held at low relative humidity were drawn through a vessel maintained at 100 % relative humidity immediately before collection, then the recovery of infectivity was increased about a 1000-fold (Table 2). This enhancement of recovery by pre-humidification has been described for other bacterial phages by Hatch & Warren (1969) and by Warren, Akers & Dubovi (1969). Table 2 also shows that a similar

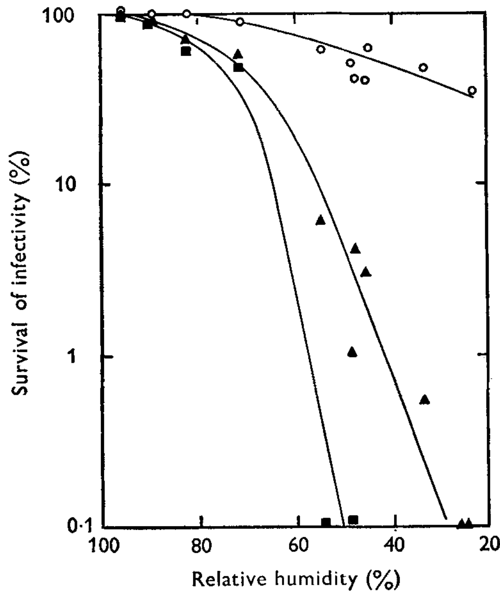

Fig. 7

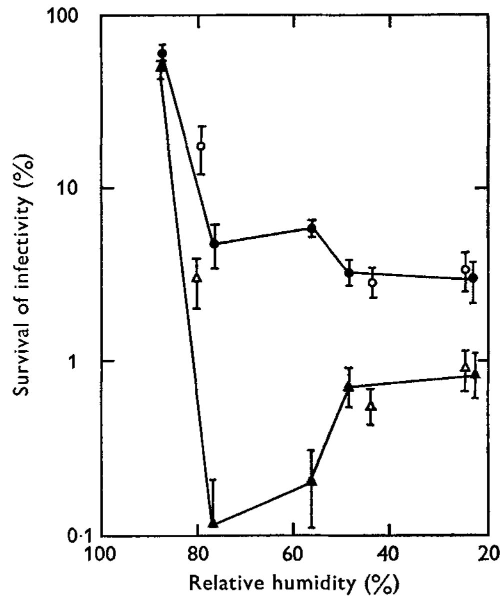

Fig. 8

Fig. 7. The survival of T7 coliphage sprayed from clarified lysates as a function of relative humidity at aerosol age: ○, 1 sec.; ▲, 5 min.; ■, 1 hr.

Fig. 8. The survival of aerosolized T7 coliphage sprayed from treated suspensions as a function of the relative humidity. ●, Desalted and deproteinized and stored as aerosol for 1 sec. after spraying; ○, desalted only and stored for 1 sec.; ▲, desalted and deproteinized and stored for 5 min.; △, desalted only and stored for 5 min.

Table 2. *The effect on recovered infectivities of the method of collection of virus aerosols after storage for 5 min. at 20 % relative humidity*

	Method of collection of virus aerosols		
Virus	Porton-raised impinger	Pre-humidification at 100% r.h. before collection by the Porton-raised impinger	May's subsonic impinger
	Recovery of infectivity (%)		
Semliki Forest virus	42±8	32±7	35±5
Langat virus	51±12	42±10	44±8
T7 coliphage	0·02±0·01	28±19	6·9±1·3
Poliovirus	3·2±1·8	29±5	16±4

higher recovery was achieved if the coliphage aerosols were collected by a subsonic liquid impinger (May, 1966) instead of the Porton-raised impinger. The higher recoveries of infectious coliphage by these collection methods could be due to a slower and more favourable rehydration of coliphages. In contrast to these effects, pre-humidification or collection by subsonic impinger resulted in slightly lower recoveries of infectivity from arboviruses in aerosols.

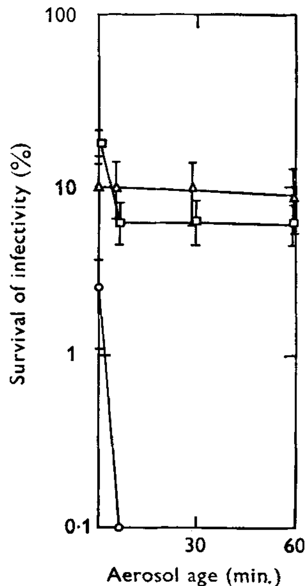

Fig. 9

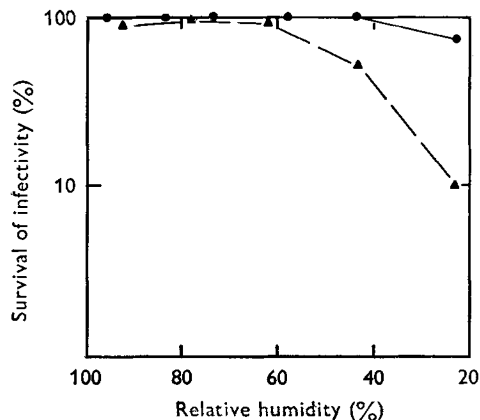

Fig. 10

Fig. 9. The survival of T7 coliphage sprayed from different suspensions and stored at 70% relative humidity as a function of aerosol age. ○, Deproteinized and desalted suspension; △, 5% (w/v) NaCl added to the desalted and deproteinized suspension; □, 5% (w/v) glucose added to the desalted and deproteinized suspension.

Fig. 10. The survival of T1 coliphage in the aerosol after 1 hr as a function of the relative humidity. Sprayed from: ●, clarified lysate; ▲, deproteinized and desalted suspension.

Table 3. *The effect of ether treatment of T1 coliphage suspension on the infectivity recovered from aerosols stored at 20% relative humidity*

	% T1 infectivity after storage for	
	10 min.	60 min.
Desalted and deproteinized suspension	18±4	9·2±2·8
Desalted and deproteinized suspension followed by extraction of lipids by peroxide-free ether	20±5	12±3

The infectivity of T1 coliphage was extremely stable in aerosols sprayed from bacterial lysates or from desalted and deproteinized fluids (Fig. 10). The high recovery of infective T1 coliphage from aerosols at low relative humidity is distinct from that of other ether-resistant viruses. It is thought that this property may be associated with the lipophilic character of T1 coliphage (Zelkonitz & Noll, 1959). To test this, aerosols were sprayed from suspensions of T1 coliphage from which extraneous lipids were first extracted by shaking

10 ml. of desalted and deproteinized suspension for 20 min. with an equal volume of peroxide-free ether. The ether layer was removed and the extraction repeated. Residual ether in the aqueous suspension was removed by evaporation before spraying the T1 coliphage. This treatment did not enhance inactivation of T1 coliphage in aerosols at 40 % relative humidity (Table 3). The good recovery of infective T1 coliphage from aerosols may thus be due to protection by adsorbed lipids, unless these are present in small amounts and not removed by the extraction.

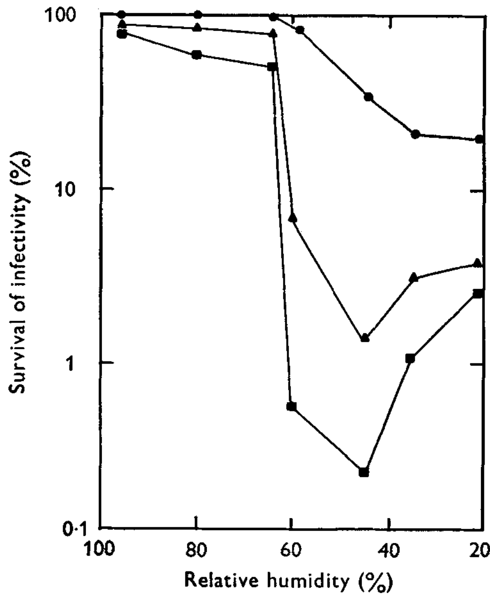

Fig. 11

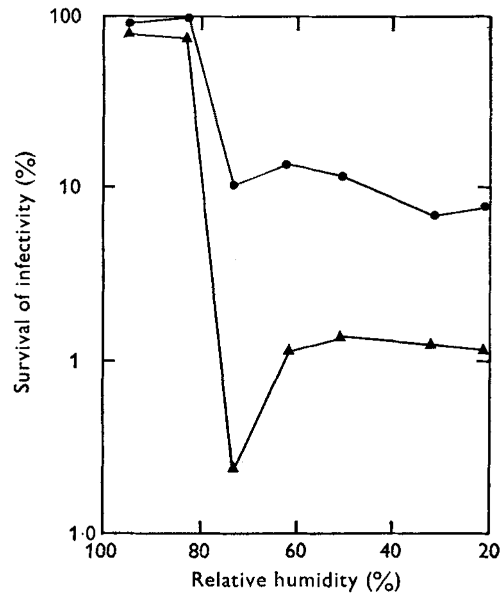

Fig. 12

Fig. 11. The influence of relative humidity on poliovirus in aerosols sprayed from clarified culture fluids. Aerosol age: ●, 1 sec.; ▲, 5 min.; ■, 1 hr.

Fig. 12. The survival of aerosolized poliovirus sprayed from a deproteinized and desalted suspension as a function of the relative humidity. Aerosol age: ●, 1 sec.; ▲, 5 min.

The survival of poliovirus in aerosols

The results obtained on the aerosol survival of poliovirus are summarized in Fig. 11 and confirm those of Harper (1961; 1963*a*, *b*) and De Jong & Winkler (1968); survival patterns are similar to those for T2 and T7 coliphages. Polyhydroxy-compounds did not enhance the aerosol survival of poliovirus but the prehumidification at 100 % relative humidity treatment did produce a tenfold increase after 1 hr storage at 40 % relative humidity. When poliovirus was sprayed from desalted and deproteinized suspensions the recovery of infectivity at 80 % relative humidity or above was not impaired, but at lower relative humidities less than 3 % survived at 5 min. (Fig. 12). Addition of soluble substances to the purified suspensions before spraying produced the same effects on survival of poliovirus as on T2 and T7 coliphage (Table 4).

The effect of atmospheric oxygen on airborne viruses

Good survival of Gram-negative bacteria at low relative humidity depends on the absence of oxygen in the atmosphere (Hess, 1965; Cox, 1966; Benbough, 1967). However, no

difference in the survival of viruses and coliphages could be demonstrated whether they were sprayed and stored in air or in oxygen-free nitrogen at 40 % and 80 % relative humidities (Table 5).

Table 4. *The effect of certain added solutes on the survival of poliovirus infectivity in stored aerosols at 70 % relative humidity*

	Aerosol age	
	1 sec.	5 min.
	Recovery of infectivity (%)	
Deproteinized and desalted suspension	11 ± 3	4 ± 3
Deproteinized and desalted suspension + 5 % (w/v) NaCl	22 ± 7	19 ± 7
Deproteinized and desalted suspension + 5 % (w/v) glucose	35 ± 8	34 ± 10

Table 5. *The survival of infectivities of viruses in aerosols at* 80 % *and* 20 % *relative humidities in atmospheres of nitrogen or air*

Aerosol age, 1 hr.

	Relative humidity			
	80 %		20 %	
	Air	N_2	Air	N_2
	Survival of infectivity (%)			
Semliki Forest virus	51 ± 10	48 ± 8	67 ± 11	52 ± 5
Langat virus	10 ± 2	11 ± 2	52 ± 8	48 ± 7
T7 coliphage	57 ± 10	61 ± 7	0·05 ± 0·02	0·05 ± 0·02
Poliovirus	53 ± 8	42 ± 12	2·5 ± 1·5	2·0 ± 1·0

DISCUSSION

The stability of the infectivity of viruses in aerosols of different relative humidities is affected by solutes present in the fluids from which the viruses were sprayed. Certain solutes have contrasting effects on inactivation in aerosols of different classes of viruses: for example, some monovalent chlorides lead to inactivation of arboviruses but to protection of T coliphages and poliovirus.

The susceptibility of viruses in aerosols to the environmental conditions must be related to the partition of bound and unbound water between virus, other constituents of the aerosol particle containing the virus, and the surrounding atmosphere. Following aerosolization there is a period of equilibration during which the concentration of solutes in the aerosol particle increases to levels which may be toxic to the virus. Therefore, the survival of viruses in aerosols may depend on the water activities of saturated solutions of the solutes in the spray fluid (Bateman, 1968). Differences between the toxic effects of LiCl, NaCl and KCl on arboviruses in aerosols may be explained in terms of the water activities of saturated solutions of these salts. The water activities of saturated solutions of these salts are 0·10, 0·75 and 0·85, respectively (Wexler & Hasegawa, 1954) and, if it is assumed that the salts are toxic in solution and non-toxic when in the solid form, it would be expected that the maximum toxic effect of LiCl, NaCl and KCl would be at relative humidity values just greater than 10, 75 and 85 %, respectively. However, maximum aerosol inactivation

of Langat virus by NaCl and KCl occurred at relative humidities below these values. This could be explained if, following equilibration of airborne particles, supersaturated solutions of NaCl and KCl, were formed. LiCl would not be expected to form crystals within the 100 to 20 % range of relative humidity that was studied. Thus, the increased virucidal effect of LiCl at lower relative humidity is probably due to its increased concentration in particles containing virus. However, salts present in the spray fluids of T coliphages and poliovirus reduced the inactivation of these viruses in aerosols. A different mechanism of virus inactivation following aerosolization probably accounts for the different effect of salts on arboviruses and on non-lipid viruses. The rehydration of aerosol particles during the collection process accounts for the considerable inactivation of non-lipid viruses but not of arboviruses. Salts and many other solutes which enhance the survival of the infectivities of T coliphages and poliovirus may do so as a result of modifying the rehydration rate during collection.

The toxic effects of salts on arboviruses in aerosols and on frozen and freeze-dried viruses may be due to a similar mechanism. Possible mechanisms of salt toxicity on frozen and freeze-dried viruses have been described by Greiff & Rightsell (1966); strong solutions have been reported to dissolve lipoprotein (Lovelock, 1957). Thus, the inactivation of arboviruses sprayed from fluids containing salts could be due to the dissolution of the lipoproteins which are present in the virus. This breakdown of the lipoprotein of viruses in aerosols may be caused by Cl^- ions displacing bound water in membrane systems (Webb, 1965). The ability of polyhydroxy-compounds to nullify the toxicity of salts towards aerosolized arboviruses might be due to such hydrophilic compounds being preferentially adsorbed at sites normally affected by salts. If the degree of lipoprotein degradation by salts depends on the structure of the lipoproteins as suggested by Lovelock, then arboviruses having different lipoproteins may be expected to have different susceptibilities to salts in aerosols. Also, since virus lipids may be determined by host of origin (Kates *et al.* 1962), viruses grown in different types of animal cells probably have different aerosol properties when sprayed from suspensions containing salts.

It is of interest to consider the stability of arboviruses in aerosols in relation to the inhalation hazard to laboratory workers. A survey by Hanson *et al.* (1967) on laboratory infections by arboviruses showed that many group A and group B arboviruses have been responsible for such infections with the highest number of incidents due to Venezuelan equine encephalomyelitis (a group A arbovirus). In laboratory conditions, aerosols containing a group A arbovirus generated from culture fluid would remain infective for a number of hr and therefore may represent a potential hazard. If results obtained for Langat virus are fairly typical of the aerosol behaviour of group B arboviruses generated from the culture fluid into the laboratory (relative humidity between 40 and 60 %), the potential hazard would probably be less. Aerosols of group A or group B arboviruses generated from fluids of low solute content may represent an even greater hazard since they have been shown to remain infective for even longer periods and, because of their smaller particle size, may penetrate deeper in the respiratory system.

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