Archives of Virology 51, 263—273 (1976)
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Survival of Airborne Influenza Virus: Effects of Propagating Host, Relative Humidity, and Composition of Spray Fluids

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With 2 Figures

Accepted February 25, 1976

Summary

Influenza A virus, strain WSN_H, propagated in bovine, human and chick embryo cell cultures and aerosolized from the cell culture medium, was maximally stable at low relative humidity (RH), minimally stable at mid-range RH, and moderately stable at high RH. Most lots of WSN_H virus propagated in embryonated eggs and aerosolized from the allantoic fluid were also least stable at mid-range RH, but two preparations after multiple serial passage in eggs showed equal stability at mid-range and higher RH's. Airborne stability varied from preparation to preparation of virus propagated both in cell culture and embryonated eggs. There was no apparent correlation between airborne stability and protein content of spray fluid above 0.1 mg/ml, but one preparation of lesser protein concentration was extremely unstable at 50 to 80 per cent RH. Polyhydroxy compounds exerted a protective effect on airborne stability.

Introduction

Survival of influenza virus in the airborne state has broad implications both in transmission of disease and aerogenic immunization with live virus. Previous studies on airborne survival of influenza have employed virus propagated and titered in embryonated eggs. The availability of cell culture systems for propagation and assay of certain strains of influenza now permits comparative aerosol studies of virus propagated in different host systems. It has been stated as a generalization that enveloped viruses, including influenza, which contain structural lipids are more stable at low relative humidity (RH) than at high, and that the opposite, maximal stability at high RH, holds for ether resistant viruses (9). Published data on the effect of RH on airborne influenza agree with this generalization, but there is a lack of agreement on survial at mid-range RH; some

reports indicate a transition between low and high inactivation rates at mid-range (11, 12, 14) but others report minimal stability at mid-range (15, 20). Of various factors affecting survival of airborne viruses, RH and composition of the spray medium are considered to be of major importance (1, 2, 4, 9). Among media components and additives that may affect survival of airborne viruses, salts, proteins, and polyhydroxy compounds have received attention (3, 4, 12, 19, 22).

We initiated this study to determine if host cell source was among factors influencing survival of airborne influenza virus. If survival were host cell dependent, virus from mammalian cell cultures, and particularly human cells, might be more typical of the agent responsible for contagious influenza than is egg-passaged virus. It is also conceivable that host cell effects on survival would be important in any potential use of live virus for aerogenic immunization. We have examined the influence of RH on airborne survival of numerous suspensions of influenza A virus strain WSN_H, propagated in embryonated eggs, primary chick embryo cell culture, as well as bovine and human cell culture lines. This approach permitted us to observe variation among different host systems, particularly with reference to instability at mid-range RH. Effects of protein concentration, dialyzable materials and polyhydroxy compounds were also examined.

Materials and Methods

Cells and Virus

A subline of MDBK cells highly susceptible to influenza virus (7) was kindly provided by P. W. Choppin. The clone 1—5C-4 Chang human conjunctival cell line (16) was obtained from the American Type Culture Collection. Primary chick embryo cells were prepared from whole minced 11-day embryos. The seed stock of cell culture-adapted WSN strain of influenza, designated WSN_H (23), was kindly provided by P. H. Duesberg. After three passages in MDBK cells, a stock virus pool, designated 0-31, was prepared in MDBK cells. Unless otherwise stated, subsequent preparations were made employing 0-31 as inoculum. A plaque purified stock was prepared after two passages in 1—5C-4 cells. An isolated plaque on 1—5C-4 cells was picked and replaqued. Virus from the second plaque was passed twice in 1—5C-4 cells to form the plaque purified pool.

Propagation of Virus

Confluent cell culture monolayers in 16-oz prescription bottles or roller bottles were washed with MEM or Dulbecco's phosphate buffered saline (PBS) and inoculated with a low multiplicity (0.002—0.01 PFU/cell) of WSN_H virus. After 1 hour adsorption, MEM was added to the infected mammalian cells and MEM with 0.1 per cent bovine serum albumin was added to the chick embryo cells. Incubation was at 37° C and virus was harvested at the peak of cytopathic effect, usually the 4th day for MDBK cells and the 2nd day postinoculation for 1—5C-4 and chick embryo cells. Eleven-day-old embryonated hens' eggs were inoculated with 0.1 ml dilute virus (1:1000) via the allantoic cavity and allantoic fluids were harvested after 2 days incubation at 37° C. Storage of virus pools was at −70° C.

Virus Assay

A modification of Kilbourne's (16) plaque assay technique in 1—5C-4 cells was employed. Prescription bottles (3-oz) were mounted in racks (21) for ease of handling including removal of a soft overlay (0.3 per cent Ionagar No. 2) and staining with crystal violet. Duplicate bottles at half-log dilutions were employed with 0.3 ml inoculum per bottle. Guinea pig or chicken red blood cells were employed in hemagglutination (HA) titrations.

Protein Determination

The method of LOWRY *et al.* (18) was used to determine protein in clarified allantoic fluids and trichloracetic acid precipitates of cell culture fluids.

Aerosol Procedures

Virus harvested from infected cell culture fluid or clarified ($10{,}000 \times g$, 10 minutes) allantoic fluid was aerosolized in a Wells refluxing atomizer operated for 6 minutes at 10 pounds/inch^2 with an effective output of 0.025 ml/minute (10). The virus suspensions were employed without dilution or additives except as noted. Each run consisted of two aerosols held simultaneously at 21° C in 208-liter dual stirred settling chambers (6). Each run employed either a single virus preparation at two different RH levels, or different preparations or additives at a single RH. Samples were taken at 1, 15, 30, and 60 minutes post-aerosolization with AGI-30 impingers operated for 1 minute (air sample volume 12.5 liters). Impinger fluid was 20 ml medium MEM with 0.1 per cent Dow Corning antifoam B. There was no significant inactivation of virus in either the atomizer or impinger fluids.

Presentation of Data

Data from each aerosol run (two chambers) was plotted on semilogarithmic paper; an example is shown in Figure 1. Theoretical 100 per cent recovery was calculated from average atomizer titer, the effective output of the atomizer, the volume of the holding chamber, and the volume of aerosol sampled by the impinger. Percentage recovery was calculated from plaque counts in duplicate bottles at half-log dilutions, and the results at each dilution (usually limited to plaque counts between 10 and 60 per bottle) were plotted as a point on the graph. Appropriate corrections were applied for removal and replacement of air during any previous sampling, but no correction was applied for physical decay of the aerosol, which was only about 10 per cent in one hour (J. HEBERT, personal communication). The best fit was estimated by eye, and the half-times ($t_{1/2}$, the time required for recovery to decrease by a factor of 2) were estimated from the lines thus drawn. The 15—60 minute line was extrapolated back to zero time to provide an estimate of the viral population exhibiting long term survival characteristics; this was designated "secondary survival". For tabular summarization (Tables 1 and 2) we used the recovery at 1 minute and the secondary survival, both expressed as percentages of calculated input, and the half-times for the 1—15 and 15—60 minute intervals. For graphical summarization (Fig. 2) the 1, 15, and 60 minute points from the best fit lines were used.

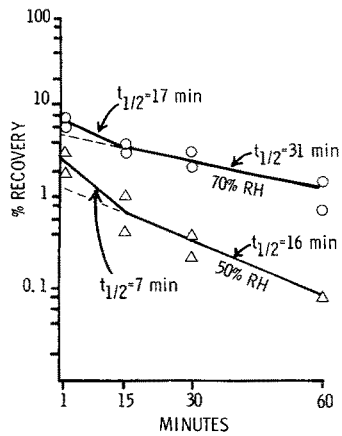

Fig. 1. Example of an aerosol run showing recovery of influenza virus at various times at two RH levels. Allantoic fluid preparation 4—14 was aerosolized and held at 50 and 70 per cent RH in paired chambers. Titers in the atomizer fluid were 5.6×10^7 PFU/ml before and 4.6×10^7 PFU/ml after aerosolization

Results

Influence of RH; Variability among Preparations

An example of a typical aerosol run with WSN_H influenza virus is presented in Figure 1. A lower recovery of virus at one minute, and a more rapid inactivation thereafter was observed at 50 per cent RH than at 70 per cent RH for that particular preparation propagated in embryonated eggs. The data suggest biphasic (or multiphasic) inactivation kinetics. The first phase(s) was a rapid initial loss of infectivity during drying and equilibration of the newly formed airborne droplets with the surrounding air. The final phase was a slower long term inactivation rate. In Figure 1 the recovery in the one-minute sample (which actually encompassed the period of one to two minutes after termination and six to seven minutes after initiation of aerosolization) was above the extrapolated secondary survival level; this resulted in a steeper slope between 1 and 15 minutes compared to the 15 to 60 minute slope. Most other, but not all, aerosol runs showed a similar relationship.

Figure 2 represents graphical comparisons of WSN_H propagated in two mammalian cell lines, primary chick embryo cell culture and embryonated eggs. These results illustrate our general findings of maximum survival at low RH, minimum survival at mid-range RH and moderate survival at high RH. There appeared to be differences among the various host sources as to the RH of minimum survival, but the validity of these differences becomes uncertain when additional preparations (not shown) are considered. For example, minimum stability at 50 per cent RH was characteristic of those MDBK preparations shown in Figure 2A, but results with a different preparation showed lower survival at 40 per cent than at 50 per cent RH. Similarly, some preparations from 1—5C-4 cells showed least stability at about 50 per cent RH whereas the example in Figure 2B was highly sensitive at 40 per cent RH, and the plaque purified preparations were least stable at 60—70 per cent RH (Fig. 2E). Variability in percentage recovery also was observed; for example, several egg-propagated preparations showed recoveries approximating 100 per cent at low RH values as compared to approximately 20 per cent in the example shown in Figure 2C. Scatter diagrams were prepared from pertinent data obtained in approximately 130 sets of observations (65 two-chamber runs) to seek indications of difference in survival attributable to host source. The diagrams for each of the four parameters (recovery at 1 minute, secondary survival, $t\frac{1}{2}$ 1—15 minute, $t\frac{1}{2}$ 15—60 minute) and for each host source showed minimal survival in RH midrange (40—60 per cent RH). However, the wide scatter of points made statistical evaluation impractical. This wide scatter could be interpreted only as variability among different preparations of virus since consistency was observed upon repeated runs with the same preparation and within paired runs with different preparations.

The plaque purified stock was employed in an attempt to assess the role of genetic heterogeneity in the variable response to RH. The results failed to implicate genetic heterogeneity, but did raise several points of interst. Whereas plaque purified stock passaged in embryonated eggs yielded RH survival patterns typical of other stocks (Fig. 2F), a parallel passage in 1—5C-4 cells (preparation 4—10) yielded atypical patterns and was unique among all our WSN_H preparations. It was highly unstable at mid- and high-range RH (50—80 per cent). Recoveries

at one minute were 1 per cent or less, and virus was rarely detectable in later samples. In the 20—40 per cent RH range, however, preparation 4—10 behaved in a typical manner. (This preparation was also unique in its low content of protein. See next section). Progeny virus from preparation 4—10 obtained by further passage in 1—5C-4 cells showed a more typical pattern of survival, as did a preparation propagated in 1—5C-4 cells after one passage in embryonated eggs (both shown in Figure 2E).

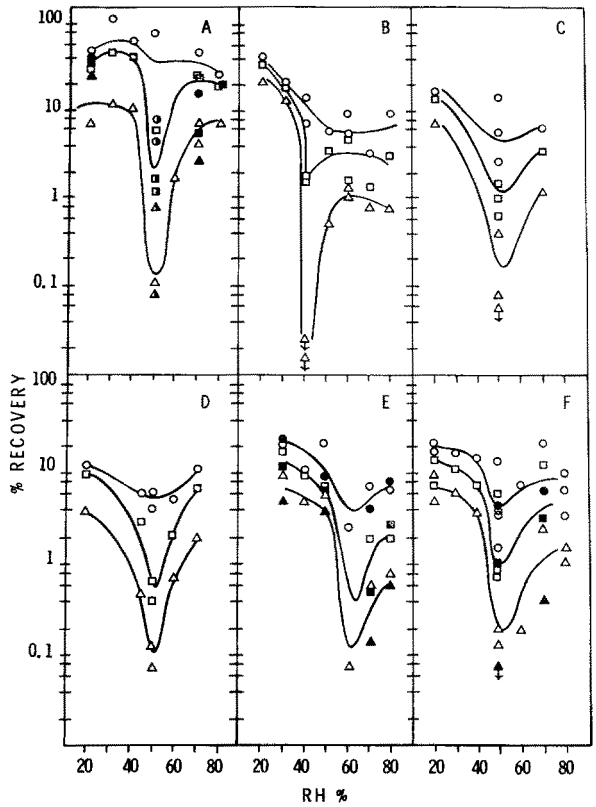

Fig. 2. Survival of WSN_H influenza virus from various sources as a function of RH. Virus propagated in: A, MDBK cells; B, 1—5C-4 cells; C, embryonated eggs; D, primary chick embryo cells. Virus from plaque purified stock propagated in: E, 1—5C-4 cells; F, embryonated eggs. One minute, 15 minutes, and 60 minutes recoveries are shown as circles, squares, and triangles, respectively; open, closed, and half-filled symbols represent different preparations

All preparations of WSN_H virus propagated in cell culture showed greater survival at high RH than at mid-range (excluding 4—10, where low recoveries precluded quantitative comparison). Minimal survival at midrange was not a consistent finding among all preparations of WSN_H virus from eggs. Table 1 presents a summary of all allantoic fluid preparations for which survival data in paired chambers at 50 and 70 per cent RH exist. Most of the preparations showed evidence of mid-range instability, i.e., lower recoveries at 50 per cent

than at 70 per cent RH. In no instance where these two RH levels were compared in the same run was there greater survival at 50 per cent RH. However in two preparations, 2—7 and 3—35, recoveries were approximately equal at 50 and 70 per cent. These two preparations represented the fourth and fifth serial passages in embryonated eggs, suggesting that serial propagations in this host may alter the RH stability spectrum. There was no evidence for this, however, at the second or third egg passage level in several other series. There was no obvious correlation between relative stabilities and protein content, initial infectivity titers, HA titers, and whether or not the stock was plaque purified.

Table 1. *Comparison of airborne survival at 50 and 70 per cent RH of preparations of WSN_H influenza virus propagated in embryonated eggs*

Preparation number	Egg passage number	Protein mg/ml	HA 1 / diln.	PFU/ml $\times 10^{-6}$	RH %	Recovery at 1 min. %	Secondary survival %	Half times, $t\frac{1}{2}$, min. 1 to 15 min. interval	Half times, $t\frac{1}{2}$, min. 15 to 60 min. interval
2—7	4	1.4	256	12	50	28	14	8	23
					70	26	13	8	23
2—26	1	1.0	32	135	50	13	13	25	25
					70	16	16	40	40
3—25	5	1.5	64	28	50	30	18	11	24
					70	27	23	17	22
4—11 (P)[a]	1	0.6	ND[b]	17	50	16	17	9	9
					70	23	23	19	19
4—14	2	1.0	64	51	50	3	1	7	16
					70	6	5	17	31
4—15	2	1.2	ND	12	50	13	11	7	8
					70	20	16	17	31
4—16 (P)	2	0.9	ND	1.5	50	10	NE[c]	10	NE
					70	18	19	15	15
4—19	1	0.9	32	44	50	15	7	9	26
					70	18	16	16	23
4—20	2	1.5	64	34	50	16	8	10	21
					70	25	20	13	20
4—21 (P)	2	0.5	32	6.7	50	4	2	8	12
					70	6	5	12	16
4—22	3	1.5	32	11	50	26	8	7	18
					70	34	20	13	32

[a] (P) From plaque purified stock
[b] ND Not done
[c] NE Not estimable—recovery too low at 60 minutes

Effects of Suspending Fluids and Additives on Airborne Stability

Protein content of most of the WSN_H influenza virus preparations was measured to determine if a relationship existed between total protein and airborne survival. Protein content of the allantoic fluids varied from approximately 0.5 to 1.5 mg/ml with no correlation with airborne survival (Table 1). To assure cell survival and virus yield, 0.1 per cent bovine serum albumin was present in the medium during virus propagation in primary chick cells, effecting a protein

concentration of approximately 1 mg/ml. Protein content of most MDBK and 1—5C-4 cell preparations (where added serum or albumin was not needed for good virus yield) ranged from 0.14 to 0.32 mg/ml. No correlation between protein content of these cell culture preparations and airborne survival was apparent. In contrast to all other cell culture preparations, the highly unstable plaque purified preparation 4—10 had a low protein content, 75 μg/ml. Airborne survival of this

Table 2. *Effects of additives on airborne survival of WSN_H influenza virus*

Preparation and source	RH %	Additives	Recovery at 1 min. %	Secondary survival %	Half-times, $t\frac{1}{2}$, min. 1 to 15 min. interval	Half-times, $t\frac{1}{2}$, min. 15 to 60 min. interval
1—95 MDBK	50	6% Inositol	34	22	26	>60
		MEM	5	5	22	22
3—22 AF	50	6% Inositol	23	23	>60	>60
		MEM	48	27	11	19
0—31 MDBK	70	6% Inositol	10	NE[a]	10	NE
		MEM	50	45	14	18
1—95 MDBK	80	6% Inositol	3	3	20	20
		MEM	26	18	17	47
0—31 MDBK	20	6% Inositol	30	15	13	>60
		MEM	50	50	>60	>60
0—31 MDBK	50	6% Sucrose	32	32	>60	>60
		0.6% Sucrose	28	20	16	42
4—3 CF	50	3% Sucrose	25	10	7	22
		MEM	7	1	4	15
4—3 CF	50	3% Sucrose	40	7	5	45
		3% Glucose	10	2	6	33
3—13 1—5C-4	40	3% Sucrose	10	9	24	56
		MEM	6	NE	NE	NE
4—11 (P)[b] AF	50	3% Sucrose	18	18	>60	>60
		MEM	4	NE	6	NE
4—11 (P) AF	80	3% Sucrose	13	13	29	29
		MEM	10	10	24	24
0—31 MDBK	70	3% Sucrose	18	9	10	31
		MEM	16	7	10	43
4—14 AF	20	3% Sucrose	32	13	16	>60
		MEM	19	19	38	38
3—13 1—5C-4	20	3% Sucrose	60	60	>60	>60
		MEM	40	40	48	48
1—94 MDBK	50	6% Sucrose	20	20	>60	>60
		DMSO[c]	12	9	16	29
4—10 (P) 1—5C-4	50	3% Sucrose	2	NE	NE	NE
		None[d]	1	NE	NE	NE
4—10 (P) 1—5C-4	70	3% Sucrose	8	2	6	28
		None[d]	1	NE	NE	NE

[a] NE Not estimable
[b] (P) From plaque purified stock
[c] Dimethyl sulfoxide
[d] Data taken from other runs; the 50 and 70 per cent RH data for 4—10 were from a single paired run

preparation at 50 per cent RH was appreciably enhanced by the addition of 0.3 mg/ml bovine serum albumin (3 per cent recovery at 1 minute, 1 per cent secondary survival, and half-times of 9 and 22 minutes for the 1—15 and 15 to 60 minute intervals respectively). Adjustment of protein concentration of other preparations was not systematically studied, but in connection with other tests, dilution with an equal volume of PBS or medium MEM was employed. No effect was noted upon dilution of allantoic fluids or of a high protein content (0.32 mg/ml) 1—5C-4 cell preparation, whereas virus recoveries were low in one run with a low protein content (0.14 mg/ml prior to dilution) 1—5C-4 preparation. Thus, a protein concentration less than 0.1 mg/ml appeared to adversely affect stability of WSN_H influenza virus at high and mid-range RH.

The possibility that pH or low molecular weight components present in allantoic fluid or in cell culture medium had an effect on airborne survival was tested. No attempt was made to grossly alter NaCl concentration. Dialysis of either allantoic fluid preparations or cell culture preparations against PBS lacking Ca and Mg appeared to slightly enhance airborne stability at mid-range RH in some cases, and have no effect in others. Restoration of Ca and Mg concentrations to those in cell culture medium had no appreciable effect. Adjustment of pH of allantoic fluid, initially 8.2, downward to 6.8 or 7.2, or upward to 8.8 or 9.1 prior to aerosolization had no appreciable effect on stability at 50 per cent RH.

Inositol has been reported to increase stability of certain airborne viruses (3, 4, 22). We found that inositol at a concentration of 6 per cent stabilized WSN_H influenza at 50 per cent RH. However, it enhanced inactivation at high RH and had little effect at low RH (Table 2). Sucrose at mid-range RH was equally or more effective than inositol. In contrast to inositol, sucrose had no deleterious effect at high RH and appeared to have a slightly protective effect at low RH. In single tests, glucose was less effective than sucrose at the same concentration, and dimethylsulfoxide did not exert an appreciable stabilizing effect. Sucrose added to the highly unstable preparation 4—10 showed a detectable protective effect at 70 per cent RH but not at 50 per cent.

Discussion

It is common practice in assessing RH effect on survival of airborne viruses to perform a series of tests on a single pool of one virus, or to compare results on single pools of each of two or more different viruses. Such practice yields rather complete information relative to those particular pools, but may fail to reveal differences among different stocks of the same virus. Such differences are readily apparent in our study employing many different preparations of a single virus. Thus, conclusions regarding airborne stability of a virus species or a group of related viruses based on studies with a single preparation appear unwarranted. Such results are useful, however, in relation to broad generalizations such as greater stability at low RH than at high RH. Our results are compatible with that generalization as applied to influenza virus; however, some preparations showed nearly equal survival at RH extremes (Fig. 2A, D). Our observation that some preparations of WSN_H influenza virus from embryonated eggs showed a transitional pattern of survival at mid-range while many others showed minimal

survival at mid-range may explain the conflicting reports of transitional (11, 12, 14) and minimal (15, 20) mid-range survival of influenza virus.

A paucity of information is available regarding effects of host or passage history on airborne survival of viruses. HEARN and coworkers (13) noted that yellow fever virus exhibited a shift to greater sensitivity at 50 per cent RH upon multiple passage in HeLa cell cultures. WSN_H influenza which had multiple passages in mammalian cells showed maximal sensitivity at mid-range RH; some evidence for loss of this mid-range sensitivity upon subsequent multiple passage in embryonated eggs was observed. Structural lipids of viruses have been implicated as a major factor in determining stability in aerosols (4, 9), and structural lipids of influenza viruses are known to vary with species of host cell (8). Other host-related structural factors in influenza viruses are a variety of surface properties (5), a carbohydrate antigen (8), and cleavage of the hemagglutinin polypeptide by proteolytic enzymes (17). With regard to the latter, a preliminary experiment indicated no relation to airborne stability; an embryonated egg preparation of WSN_H exhibiting instability at 50 per cent RH, and one that was relatively stable, had essentially identical patterns of HA subunits upon polyacrylamide gel electrophoresis. Our observations make it clear that large variations in stability can occur among different preparations of a single strain of influenza virus. Because the variations among different preparations from one host source overlap those of another we cannot definitively conclude that the species of host cell plays a major role in airborne stability.

Composition of the medium from which an aerosol is generated grossly affects airborne stability of viruses (1, 2, 3, 4, 9, 12, 19, 22). These effects must ultimately relate to the structure of the virion or its genome. They may directly (in relation to considerations in the preceding paragraph) or casually (in the experimenter's choice of culture conditions or manipulation of media) relate to the host source. Concentration of dissolved solids affect the size of airborne particles and both the quantity and nature of the solids affect the degree of hydration at any given RH (10). A factor having a marked effect on airborne survival of viruses, namely salt content (3, 4, 12), was avoided in our studies by maintaining approximately physiologic levels of salts. It is common practice in virus aerosol studies to employ suspensions with relatively high protein content, either from additives or from serum in the growth medium. In some studies protein content has received attention in relation to airborne stability (3, 4, 12, 19). BENBOUGH (3, 4) reported that removal of protein from Langat and Semliki Forest virus suspensions greatly reduced airborne survival at high RH. Our results suggest that a critical level of protein (approximately 0.1 mg/ml) in the aerosolized fluid appreciably affected airborne stability characteristics of WSN_H influenza. Shifts in pH of about 1 unit, and dialysis against PBS to remove low molecular weight components revealed no appreciable changes in airborne stability. Similarly, addition of normal allantoic fluid to cell culture preparations had no effect. Since the composition of allantoic fluid from infected eggs probably differs from that from uninfected eggs, we considered the possibility that some protective substances may have been present in those preparations not exhibiting minimal survial at mid-range RH. Experiments employing ultraviolet treated preparations were inconclusive but suggestive that some protective material may have been present.

Polyhydroxy compounds have been reported to exert a protective effect on airborne virus stability, and their relation to the state of hydration has been discussed (3, 4, 22). We confirmed the protective effect of inositol at mid-range RH, but found it to be deleterious at high RH. Sucrose, on the other hand, was equally or more protective than inositol, and showed no such deleterious effect. The reason for this difference was not apparent, but the difference in molecular size may have played a role.

It is obvious from the work of others, and extended by our own, that many complicated and sometimes interrelated factors are involved in survival of airborne viruses. Different technical approaches employed by various investigators make comparisons difficult. Further, by their nature, the methods and techniques employed in the laboratory create conditions which are artificial in relation to actual airborne transmission of disease, efficacy of aerogenic immunization, and laboratory biohazards. Thus, generalizations and extrapolations to other virus systems must be viewed with these complexities in mind.

Acknowledgments

We thank James Hebert and John Erpilla for able assistance and expertise in aerosol technology, and Gloria Tung for able assistance in various aspects of the study.

This research was supported by the Office of Naval Research.

References

1. AKERS, T. G.: Survival of airborne virus, phage and other minute microbes. In: DIMMICK, R. L., AKERS, A. B. (eds.), An Introduction to Experimental Aerobiology, 296—339. New York: Wiley—Interscience 1969.
2. AKERS, T. G.: Some aspects of the airborne inactivation of viruses. In: HERS, J. F. P., WINKLER, K. C. (eds.), Airborne Transmission and Airborne Infection, 73—81. Utrecht: Oosthoek Publ. Co. 1973.
3. BENBOUGH, J. E.: The effect of relative humidity on the survival of airborne Semliki Forest virus. J. gen. Virol. **4**, 473—477 (1969).
4. BENBOUGH, J. E.: Some factors affecting the survival of airborne viruses. J. gen. Virol. **10**, 209—220 (1971).
5. BORECKY, L., LACKOVIC, V., RATHOVA, V.: Surface properties of the egg and mouse lines of A1 influenza virus. J. Immunol. **95**, 387—395 (1965).
6. CHATIGNY, M. A., WOLOCHOW, H., LEIF, W. R., HEBERT, J.: The toxicity of nitrogen oxides for an airborne microbe: Effects of relative humidity test procedures and containment and composition of spray suspension. In: HERS, J. F. P., WINKLER, K. C. (eds.), Airborne Transmission and Airborne Infection, 94—97. Utrecht: Oosthoek Publ. Co. 1973.
7. CHOPPIN, P. W.: Replication of influenza virus in a continuous cell line: High yield of infective virus from cells inoculated at high multiplicity. Virology **39**, 130—134 (1969).
8. COMPANS, R. W., CHOPPIN, P. W.: The structure and assembly of influenza and parainfluenza viruses. In: MARAMOROSCH, K., KURSTAK, E. (eds.), Comparative Virology, 407—432. New York: Academic Press, Inc. 1971.
9. DE JONG, J. C., TROUWBORST, T., WINKLER, K. C.: The mechanism of virus decay in aerosols. In: HERS, J. F. P., WINKLER, K. C. (eds.), Airborne Transmission and Airborne Infection, 124—130. Utrecht: Oosthoek Publ. Co. 1973.
10. DIMMICK, R. L.: Production of biological aerosols. In: DIMMICK, R. L., AKERS, A. B. (eds.), An Introduction to Experimental Aerobiology, 22—45. New York: Wiley—Interscience 1969.

11. HARPER, G. J.: Airborne microorganisms: Survival tests with four viruses. J. Hyg. (Camb.) **59**, 479—486 (1961).
12. HARPER, G. J.: The influence of environment on the survival of airborne virus particles in the laboratory. Arch. ges. Virusforsch. **13**, 64—71 (1963).
13. HEARN, H. J., JR., SOPER, W. T., MILLER, W. S.: Loss of virulence of yellow fever virus serially passed in HeLa cells. Proc. Soc. exp. Biol. Med. **119**, 319—322 (1965).
14. HEMMES, J. H., WINKLER, K. C., KOOL, S. M.: Virus survival as a seasonal factor in influenza and poliomyelitis. Nature **188**, 430—431 (1960).
15. HOOD, A. M.: Infectivity of influenza virus aerosols. J. Hyg. (Camb.) **61**, 331—335 (1963).
16. KILBOURNE, E. D.: Plaque formation by influenza viruses, In: HABEL, K., SALZMAN, M. P. (eds.), Fundamental Techniques in Virology, 146—160. New York: Academic Press, Inc. 1969.
17. LAZAROWITZ, S. G., GOLDBERG, A. R., CHOPPIN, P. W.: Proteolytic cleavage by plasmin of the HA polypeptide of influenza virus: host cell activation of serum plasminogen. Virology **56**, 172—180 (1973).
18. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193**, 265—275 (1951).
19. RECHSTEINER, J., WINKLER, K. C.: Inactivation of respiratory syncytial virus in aerosol. J. gen. Virol. **5**, 405—410 (1969).
20. SHECHMEISTER, I. L.: Studies on the experimental epidemiology of respiratory infections. III. Certain aspects of the behavior of type A influenza virus as an airborne cloud. J. inf. Dis. **87**, 128—132 (1950).
21. SPENDLOVE, R., TAYLOR, F. L.: A rack for expediting the manipulation of tissue culture bottles with an application to the viral plaque technique. Amer. J. clin. Pathol. **40**, 34—37 (1963).
22. WEBB, S. J., BATHER, R., HODGES, R. W.: The effect of relative humidity and inositol on airborne viruses. Canad. J. Microbiol. **9**, 87—92 (1963).
23. ZITCER, E. M., BRUENING, G., AGRAWAL, H. O.: The multiplication of an influenza virus strain in a continuous line of mammalian cells. Arch. ges. Virusforsch. **20**, 137—141 (1967).

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Received January 30, 1976