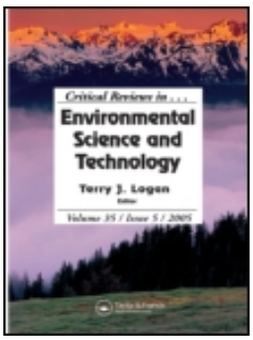

Critical Reviews in Environmental Science and Technology

ISSN: 1040-838X (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/best19

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To cite this article: Syed A. Sattar , Mohammad Khalid Ijaz & Charles P. Gerba (1987) Spread of viral infections by aerosols, Critical Reviews in Environmental Science and Technology, 17:2, 89-131, DOI: 10.1080/10643388709388331

To link to this article: https://doi.org/10.1080/10643388709388331

Published online: 09 Jan 2009.

SPREAD OF VIRAL INFECTIONS BY AEROSOLS

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I. INTRODUCTION

Viruses are responsible for a variety of diseases in humans and animals. It has been estimated that nearly 60% of the cases of all infections in man are due to viruses.[1] Although no such estimates are available for animals, viral infections in species of economic importance are known to result in significant losses every year throughout the world. Only a limited number of safe and effective vaccines are presently available for the prevention and control of such diseases. The number of drugs available for their treatment is even more limited. Therefore, at the present time, efforts to protect against the health and economic impact of viral diseases rely heavily on personal hygiene and public and veterinary health measures. The success of any such measures, in turn, largely depends on our knowledge of how different viral diseases spread in nature.

The concept of airborne contagion is an ancient one, but serious study of the spread of infections by the airborne route had its beginnings in the 1930s. It received a further impetus during World War II because of the problems of respiratory illness in military populations. As a result of the investigations conducted during the past five decades, it is now well-established that a variety of infectious diseases, including many caused by viruses, are able to spread by the airborne route. However, in instances where contaminated vehicles such as fomites, water, and food have been implicated in virus transmission, the potential importance of air as a vehicle may have been overlooked. In theory, almost any virus could spread through the air, but if air is to be a major vehicle for the spread of any particular virus, then the virus must be able to survive the process of aerosolization and to persist in the airborne state long enough to allow transmission to a susceptible host.

A great deal of work has been conducted to determine which factors promote or retard the survival of human and animal pathogenic viruses in air, and what preventive and control measures are likely to be effective in safeguarding against airborne infections. It is the purpose of this review to critically evaluate the available information with regard to (1) the methodology used to study viral aerosols; (2) the influence of

various environmental factors on virus survival in air; (3) experimental transmission of viral infections by the airborne route; and (4) documented airborne spread of viral diseases in humans and animals.

II. AEROSOLS AND THE SPREAD OF INFECTIOUS DISEASES

Aerosols are dispersions in air of particles of a variety of sizes. The larger of these particles rapidly settle out, but particles of smaller size can remain suspended in air for longer periods. If the air were to be perfectly still, it would take a 100 μm diameter particle 10 sec to fall through the height of an ordinary room (about 3 m); particles with diameters of 40, 20, and 10 μm would require 1, 4, and 17 min, respectively, to settle out under the same set of conditions.[2,3] Under real conditions, the time during which aerosol particles remain suspended and the distance which they can travel from the point of their generation are greatly influenced by airflow and turbulance.

Many common and natural activities in the domestic, work, or animal husbandry environments regularly result in the generation of aerosols from microbially contaminated liquids or the resuspension in air of previously dried infectious material. For example, sneezing, coughing, and even speaking by persons carrying viruses in their mouth and respiratory tract frequently lead to the aerosolization of viruses.[4,5] The particles produced during sneezing and speaking (particularly when pronouncing sibilants) are generally larger and most of them rapidly settle out of air. Coughing, on the other hand, is known to produce more small-particle aerosols which are potentially better suited for the airborne spread of viral infections.[5]

Upon aerosolization, and depending on the level of relative humidity (RH) and atmospheric temperature, most of the water from aerosolized particles of small size evaporates almost immediately. This leaves behind a residual particle which may contain organic and inorganic materials as well as biological agents. Residual particles of this type (usually <5 μm in diameter) are referred to as "droplet nuclei",[6] and, if the biological agents in them are not damaged by the drying process, they are then potentially infective for susceptible host species. Under conditions of normal aerial turbulence, droplet nuclei can remain airborne for prolonged periods of time. Inhalation of air containing these particles can lead to their retention in the respiratory tract.[7-10]

Larger particles containing infectious agents, which tend to settle out immediately, can also be important in disease transmission. If the infectious agents in them manage to survive the initial process of aerosolization and subsequent drying on the surface where they have settled, direct or indirect contact of susceptible hosts with such surfaces could lead to the spread of virus infections. The possible ways in which microbial aerosols can transmit infections are schematically represented in Figure 1. Since true airborne transmission of infectious diseases is generally considered to occur through the inhalation of droplet nuclei, this review will place particular emphasis on the role of such particles in the spread of viral diseases. However, it should be noted that, in conditions of reduced gravity during space flight, larger-size aerosol particles may also be inhaled and may increase the risk of airborne infection.[11]

III. PARTICLE SIZE AND VIRUS RETENTION IN THE RESPIRATORY TRACT

The particle size of virus-containing aerosols is important not only for their ability to stay suspended in air, but also as the principal factor determining the site and extent of retention of inhaled particles in the respiratory tract.[12,13] Therefore, some knowledge of the particle diameters of viral aerosols is considered essential in understanding

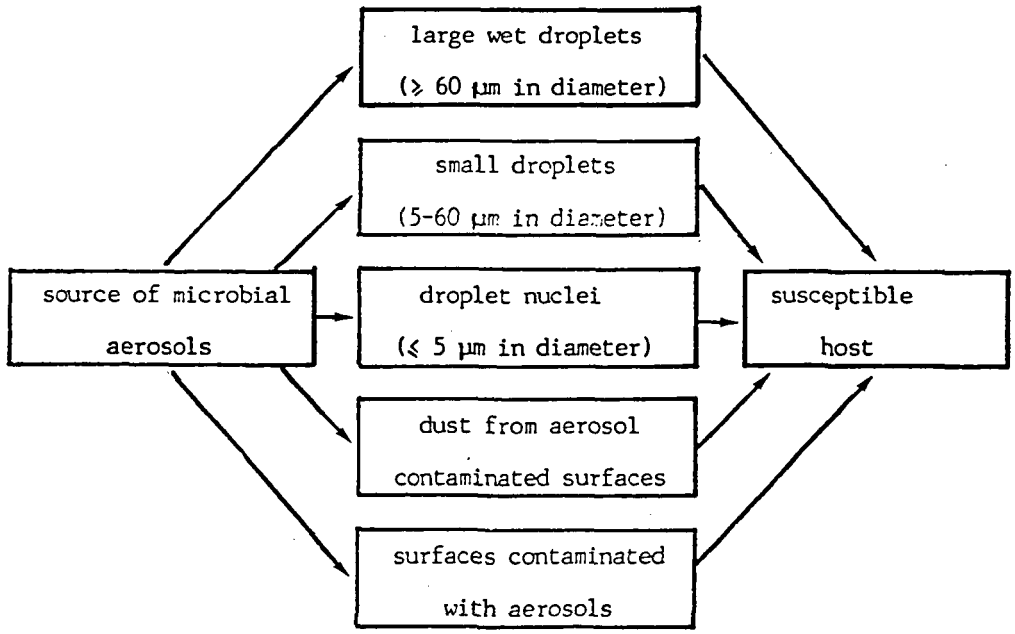

FIGURE 1. Possible ways of spread of infections through microbial aerosols.

airborne transmission of viral infections. From the point of view of infectious disease spread by the airborne route, particles of particular importance fall in the size range of 0.1 to 60.0 μm in diameter.[14]

A human adult breathes between 10,000 and 20,000 *l* of air per day.[15] Such air may contain particles of many types and sizes.[9] Increased ventilation due to exercise may also increase the deposition of inhaled aerosols.[16] The greatest degree of deposition in the alveoli of human lungs occurs when the inhaled particles are in the 1 to 2 μm range; it decreases to a minimum for particles of 0.25 μm. For particles below 0.25 μm, alveolar deposition again increases due to Brownian movement.[8,9,12,17,18] Fuchs[19] observed that 82, 28, and 51% of the particles 1.0, 0.1 to 0.3, and 0.03 μm, respectively, were retained in alveoli or alveolar passages. The entry of hygroscopic particles into the moisture-laden respiratory tract results in an increase in their diameter, and this in turn could affect their site of deposition.[20] The factors that determine the deposition of hygroscopic aerosols in the respiratory tract have been reviewed recently.[21]

Here it must be noted that larger particles, which are deposited mainly in the upper portions of the respiratory tract, can be translocated by mucociliary action and ingested.[22] This could conceivably result in the spread of certain infections of the gastrointestinal (GI) tract by the airborne route.[23] Existence of such a mechanism is further supported by evidence from studies of inhaled antigens.[24]

IV. GENERATION, STORAGE, AND COLLECTION OF VIRAL AEROSOLS

In order to study the ability of individual viruses to survive as aerosols under specific conditions, it is essential to be able to produce experimentally viral aerosols of a suitable size, to store them while they age, to sample them for the infectious virus content, and, where possible, to assess the size of aerosol particles formed.

The most popular device for the experimental generation of viral aerosols is the

Collison nebulizer,[25] but the DeVilbiss, Vaponefrin, or other nebulizers can also be used for this purpose.[7,26-28] In the three aerosol generators mentioned above, the virus suspension is forced through small nozzles and mixed with compressed air. As the air expands, the suspension is broken into airborne particles of a wide size range. The larger of these are trapped by a baffle and refluxed into the virus suspension. Particles of smaller size, a major proportion of which are less than 5 μm in diameter, can remain airborne for longer periods and may be carried out of the nebulizer in the air jet. Particles of this size are important in the transmission of airborne infections because of their potential for retention in the respiratory tract. Factors such as the pressure and relative humidity of the air, and the viscosity of the suspending medium affect both the size and number of aerosol particles generated by these nebulizers.[25] Berke and Hull[29] developed a nebulizer which automatically compensates for changes in the concentration and volume of a solution during aerosolization by adjustment of the temperature and pressure of the atomizing air jet.

Aerosols produced by these devices are of a suitable size for studying the aerosol stability of viral suspensions, however, some practical difficulties are apparent in handling very small volumes of material, natural suspending media with high viscosities, or viral suspensions with a low titer of infectious virus. Observed losses of infectious virus after nebulization can be considerable (often 10- to 100-fold), but it is not always clear how much of this loss is due directly to forces exerted upon the virus during nebulization, and how much may be due to other factors associated with the suspending medium or physicochemical changes in the aerosolized particles immediately after nebulization.

In order to study the aerosol stability of viruses, it is essential to properly collect the experimentally generated aerosols for analysis. Several devices have been developed to provide information on the particle size and concentration of materials in the aerosols under study. The most commonly used of these in the study of virus aerosols is the all-glass impinger (AGI),[30] which collects the aerosols in the sampled air in a liquid medium. The AGI has been used in studying the aerosol stability of the following types of vertebrate viruses: Colorado tick fever, vesicular stomatitis (VSV), neurovaccinia, encephalomyocarditis (EMC),[31] Rous sarcoma,[34] adenovirus types 4 and 7, parainfluenza,[33] Newcastle disease (NDV),[34] infectious bovine rhinotracheitis (IBV),[34,35] bovine parainfluenza type 3, bovine adenovirus type 3,[36,37] rinderpest,[38] polio virus type 1 (Sabin),[39-42] rhinovirus type 14,[39,40] human coronavirus 229E,[39,42] calf rotavirus,[41] human rotavirus,[43] and simian rotavirus.[44] Here it should be noted that AGI is considered relatively inefficient for the collection of viral aerosols where a high proportion of the droplets is in the very small size range.[45] Such small particles have a tendency to escape entrapment in the aerosol collection fluid.

May and Druett[46] have described a simple device called a preimpinger, which, when fitted to the front of an impinger, divides the total aerosol sample into two particle size fractions by means of size-selective impingment into liquid. The cutoff between the two fractions is set at 4 μm to simulate nasal penetration. They concluded that the particle retention by the preimpinger is similar to that of the nasal passages, while the material in the backing impinger is similar to that reaching the lungs.

Other devices for the collection of aerosols are named after the type of opening through which the aerosol is sampled: (1) the slit sampler in which airborne particles pass through a narrow slit and are then impacted onto a slowly revolving dish containing a solid or semisolid collecting medium;[47] (2) the sieve sampler where the airborne particles accelerate through small holes and impact on the collection surface;[47] and (3) the stacked-sieve or Anderson sampler which consists of six sieves stacked one on top of the other[48] (each sieve contains holes of a different diameter, and this sampler thus

permits the collection of six groups of airborne particles separated on the basis of the particle diameters).[49-53] However, the use of solid (filter membranes) or semisolid (agar or gelatin) materials for the impaction of the aerosols to be collected make them less suitable for working with viral aerosols. In a limited number of studies on the aerobiology of viruses, the Anderson sampler has been adapted to estimate the size range of the viral aerosol.[49,54]

The large-volume air sampling (LVAS) devices, which can sample up to 10 m^3/min, are a relatively recent development in the study of airborne infectious agents. Spendlove and Fannin[55] have recently reviewed the literature on these, and other, aerosol collection devices.

Fluid collection media from the AGI or LVAS are used for virus assay by standard cell culture techniques. Infectious virus can be eluted from solid or semisolid media before assay, or cell cultures can be overlaid directly on the collection plates.[56] Although direct inoculation into cell culture for virus assay has been used,[56,57] it cannot be reliably carried out, even in the presence of antibiotics, when the virus aerosol may be heavily contaminated with bacteria and fungi. Low levels of purified virus may, however, be directly assessed in cell culture.[57]

In addition to various types of aerosol generators and collectors, suitable pieces of equipment are available for the transportation and aging of viral aerosols. These provide containment for experiments on infectious aerosols where the aerosolized material is maintained in the air for extended periods of time; they can also be used as a safe means of transporting infectious virus aerosols to susceptible experimental hosts. The Henderson apparatus[58,59] is most commonly used for aerosol challenge studies in animals. It can supply a continuous source of the infectious agent at a constant cloud density under controlled conditions of RH and temperature. By placing appropriate test animals in the aerosol cloud, they can then be exposed to the infectious agents for susceptibility and transmission studies. McVicar and Eisner[60] have reported a relatively simple and inexpensive means of exposing cattle of all ages to virus aerosols. They used foot-and-mouth disease virus and a plastic-covered greenhouse chamber. Another relatively simple aerosol exposure chamber for pigs and other species has been described recently.[61]

In experimental transmission studies where whole animals are exposed to an aerosol cloud, it is difficult to distinguish between virus inhaled as an aerosol and virus ingested from coat grooming or other contact with surfaces on which virus may have been deposited. However, such unrestrained animals will experience a minimal stress. On the contrary, restrained animals may only be infected by inhalation of virus particles, but the effect of stress on susceptibility to virus infection is unknown. Once respiratory transmission has been shown for a particular virus-host system, it seems likely that a more reliable prediction of the importance of viral aerosols in disease transmission can be obtained by whole animal exposure.

Survival characteristics of any given infectious agent can be studied in an aerosol maintenance chamber known as the toroid or rotating drum.[62] Goldberg[63] has presented in detail those factors that govern the behavior of aerosolized particles in the rotating drum. This device permits the maintenance of an infectious agent in an airborne state over a period of days so that the rate of its biological decay can be measured under controlled conditions of RH and temperature. The continuous rotation of the drum at a predetermined rate minimizes the loss of the aerosol due to physical decay or "fallout" on the walls of the drum. Nevertheless, it is usual to monitor and compensate for the physical decay of the aerosol particles by means of a tracer. Bacterial spores have often been used for this purpose,[64] but hazards associated with their use and the possible loss of spore viability during the generation and aging of aerosols[65]

make them less desirable for this purpose. Fluorescent dyes, such as sodium fluorescein or rhodamine, are now commonly used as physical tracers because they are inexpensive and sensitive tools for their measurement are readily available. However, it is essential to first determine that such dyes are not detrimental to the survival of the viruses under test. The suitability of such dyes as tracers has been further confirmed by direct comparison with a radiolabeled virus suspension.[54]

May and Druett[66] and Druett[67] have described an interesting microthread technique for studying the viability of microbes in a simulated airborne state. Microorganisms, captured on ultrafine spider threads, may be subjected to any environment of interest for extended periods of time, and the loss of viability of the organisms may then be determined. The validity of this technique has been tested by comparison with more conventional aerosol methodology using bacterial aerosols. Although some differences were observed in the biological decay of the bacteria tested,[66] the general correspondence of the data suggests that the spider thread technique has potential for wider application. However, the anchoring of small diameter particles to spider threads also represents a form of surface attachment. Further studies would be required to determine if such attachment in any way influences the pattern of biological decay of viruses. Other limitations of this technique have been discussed by Spendlove and Fannin.[55]

During experimental aerosol generation, a virus suspension is subjected to a variety of forces at the air-water interface which may lead to the loss of infectivity. This has led to a distinction by some investigators between a loss of virus due to spraying and a loss during storage in the airborne state. Although such a theoretical distinction is extremely valid, in most experimental systems used to study airborne virus survival it is rather artificial because of practical difficulties in distinguishing these two separate components of virus inactivation. An alternative experimental approach involves allowing an equilibration period during which the sprayed virus is allowed to stabilize in the storage chamber before sampling the aerosol for infectious virus. Results are then normalized, not to the original virus titer in the spray fluid, but to the first sample collected after equilibration. Different groups of investigators have used not only different methods of expressing results, but different lengths of time for determining initial loss due to spraying or equilibration periods of different lengths. This frequently makes it very difficult to compare results from different studies. Since disease transmission by airborne viruses requires that they remain infectious in the airborne state long enough for effective transmission, this review will concentrate on loss of virus due to storage rather than spraying. Furthermore, losses due to natural methods of aerosolization are completely unknown, and may in no way correspond to those observed during experimental nebulization.

V. FACTORS GOVERNING VIRUS SURVIVAL IN THE AIRBORNE STATE

Factors that influence the survival of airborne viruses include (1) atmospheric temperature and RH, (2) nature and composition of the spray and collection fluids, (3) atmospheric gases, and (4) irradiation.[68,69] The following shows how the above-mentioned factors affect a variety of airborne vertebrate viruses. However, it should be noted that studies on the aerobiology of viruses in general show a wide variation in their experimental design and presentation of results. This may account for some of the apparent discrepancies observed in the effects of environmental factors on the airborne survival of even closely related viruses, and sometimes makes it difficult to directly compare the findings of different investigators.

A. Temperature and RH

These two factors, which often act in combination, are perhaps the most important in determining how well viruses survive in the airborne state. The study of their influence on the infectivity of viral aerosols also provides clues to understanding how the climate affects the occurrence of many viral diseases. Table 1 presents available data on the survival of various airborne viruses: the information has been abstracted from data published between 1943 and 1986. Following are some general remarks based on the findings of these studies.

As is true in other components of the environment, the capacity of viruses to survive in the airborne state is, in general, inversely proportional to the temperature of the air. Some investigators also postulated that there was a simple correlation between the chemical composition of a given virus and the influence of RH on its airborne survival.[70] It is generally believed that lipid-containing viruses survive better at low levels of RH and that the high RH levels are more conducive to the airborne survival of lipid-free viruses.[71-73] Although many of the viruses tested obey this rule, notable exceptions have been reported.

Under certain experimental conditions, some types of viruses have been found to survive well at high and low RH levels, but were sensitive to inactivation at the mid-RH range. Examples of such viruses are VSV,[31] polio and Langat viruses,[74] mengovirus,[75] two types of caliciviruses,[72] calf rotavirus,[76] and influenza virus.[77] In contrast to this, other studies with rotaviruses of human and animal origin[41,43,44] and a human coronavirus[42] have shown them to survive best in the airborne state at 20°C when the RH is kept at 50%. Furthermore, certain enveloped viruses such as Rous sarcoma virus[78] and infectious bovine rhinotracheitis virus[34] have been found to survive best at high RH, whereas others appear to be little affected (Rift Valley Fever virus,[79] pigeon pox [78]) by RH levels in the range tested.

Humidification of aerosols immediately prior to their collection has been shown to increase the recovery of infective particles of certain types of viruses.[74,80-86] This indicates that rehydration of airborne virus particles, resulting from the prehumidification, leads to their reactivation. Infective yields of certain other vertebrate virus aerosols, such as vesicular exanthema virus,[87] remained unaffected by prehumidification. Prehumidification has also been shown to decrease the efficiency of recovery of some types of viruses.[86]

It is not yet clearly understood by what mechanism(s) air temperature and RH promote or retard the survival of airborne viruses. Akers[88] has proposed that the humidity-dependent inactivation of aerosolized viruses occurs immediately after they are sprayed, and, once established, does not drastically change with the aging of the aerosol. Akers[88] also suggests that the effect of air temperature is secondary in the inactivation of airborne viruses. Our own studies with a human coronavirus sprayed from tryptose phosphate broth have shown that atmospheric temperature has a pronounced effect on the way this virus survives in air. When the aerosols of this virus were held at 20°C, its half-life at 80% RH was only 3 hr. However, by simply reducing the air temperature to 6°C, the half-life of this virus at 80% RH jumped to nearly 87 hr.[42] The ability of low temperature to overcome the effect of RH on an enveloped virus such as coronavirus suggests that decreased fluidity of the lipid bilayer may be involved in restricting access of inactivating factors to the virus nucleic acid or protein components. Low temperature also favors more rigid orientation of water and its solutes around aerosolized viruses at high humidity.

B. Nature and Composition of Spray Fluid

It is well established that the composition of the fluid from which the virus is aerosolized can greatly influence its subsequent airborne stability.[32,35-37,74,83,88-92] Therefore,

Table 1
EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF AEROSOLIZED VIRUSES

Virus(es)	Temperature (°C)	Relative humidity (%)	Period of aerosol aging	Remarks	Ref.
Influenza A	27—29	23, 48, 89	24 hr	Experiments were performed in a room of 800 ft³ capacity; low RH experiments were performed in winter, and high RH was generated by vaporizing steam into the room; the virus survived best at 23% RH	226
Influenza A (W.S. strain)	?	30—70	?	Highest levels of airborne infectious virus were recovered at the 2 RH extremes (32 and 68%)	227
Influenza (PR8), polio virus type 1 (CSL)	?	0-100	2 min	For influenza virus, inactivation rate was high at 50—90% RH and low at 15—40% RH; for polio virus, the reverse was true; they emphasized the role of RH indoors as an important factor in the seasonal fluctuation of outbreaks due to these viruses	70
Vaccinia, influenza (PR8), Venezuelan equine encephalitis (VEE), and polio type 1 (Brunhilde)	7—11.5, 20—24, 30—33.5	17—36, 48—65, 80—86	up to 23 hr	For all the virus types studied, survival at all RH ranges tested was better at the lower than at the higher temperature; polio virus survived best at high RH levels, whereas the other 3 viruses tested survived best at low RH levels	90
Yellow fever and Rift Valley fever	25	50, 85	1 hr	Both viruses were highly stable as aerosols; no significant effect of humidity, in the range tested, was observed either on initial virus concentration or decay rate	79

Pigeon pox and Rous sarcoma	?	0—100	5 hr	Pigeon pox virus was found to be stable in aerosols and was little affected by RH. Rous sarcoma virus was extremely sensitive to RH and survived best at RH levels above 70%; inositol at a final concentration of 6.0% in the spray fluid was able to prevent inactivation of Rous sarcoma virus at RH levels below 70%	32
Measles (Edmonston strain)	20—21	10—100	?	The virus was sprayed in a temperature- and RH-conditioned room; best survival was observed below 40% RH; indoor RH was thought to be an important factor in the seasonal variations of outbreaks due to the virus	228
Encephalomyocarditis (Mengo, Maus Elberfeld, and Columbia SK)	16, 26	5—95	6 hr	At 16°C, virus inactivation during the first 5 min after spraying was maximal at high (>80%) and low (<5%) RH; at 26°C, mid-range RH (40—60%) was the most deleterious to virus survival; inactivation patterns of the virus during aerosol storage were found to be similar to other small RNA viruses such as polio	229
Newcastle (NDV), infectious bovine rhinotracheitis (IBR), vesicular stomatitis (VSV)	4, 23, 37	10, 35, 90	90 min	When stored at 23°C, NDV and VSV survived best at 10% RH, whereas IBR survived best at 90% RH; NDV was shown to survive equally well at 23 and 37°C; this was attributed to increased resistance of the virus to thermal inactivation; at 4°C and 10% RH, NDV showed no decay	34
Adenovirus types 4 and 7 and parainfluenza type 3	23.7	20, 50, 80	6 hr	Both types of adenovirus were most stable at 80% RH, whereas parainfluenza was most stable at 20% RH	33
Polio virus type 1 (strain LSc2ab)	20	0—100	1 hr	Virus survival was high below 35% and above 70% RH, but low in the range 40—60% RH; they believed that denaturation of viral RNA caused the inactivation of airborne polio virus	230

Table 1 (continued)
EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF AEROSOLIZED VIRUSES

Virus(es)	Temperature (°C)	Relative humidity (%)	Period of aerosol aging	Remarks	Ref.
Influenza A (6 human and 8 avian strains)	26.4	75	20 min	Strains of avian origin were found to have greater resistance to decay in airborne state	231
Semliki Forest	22	20—90	24 hr	Inactivation of the airborne virus was found to be maximal at high RH (84—90%) and decreased gradually as RH decreased; removal of salts from the spray fluid resulted in improved survival over the whole range of RH tested; extraneous protein was essential for survival at high RH and polyhydroxy compounds protected the virus well at low RH	98
Polio and encephalomyocarditis (EMC)	20	70—90	?	Inferred that virus inactivation at 40% RH is due to deterioration of the protein coat	232
Variola (Yamada) and yellow fever (Asibi)	26.7	30, 50, 80	1 hr	Variola virus survived better than yellow fever virus at all RH levels tested; biological decay rates not affected by RH	233
Adenovirus type 12	28—29.5	32, 51, 89	20 min	The virus was found to survive best at the highest level of RH tested (89%)	234
VEE	−40 to +49	18—90	1—2 hr	The biological decay rate of airborne VEE was not markedly affected in the temperature range −40—24°C at any RH tested (18—90%); however, at 49°C, a significant increase in decay rate was observed	235
Foot-and-mouth disease (O,BFS 1860)	19—22	20—80	1 hr	The virus was found to be stable at and above 55% RH	96
Foot-and-mouth disease (8 different strains tested)	18—23	10—100	1 hr	The virus showed maximum survival at and above 60% RH; at low RH, survival of the A strain was about 10-fold higher than for either the O or C strains	94

Influenza A, (human, avian, swine, and equine strains)	21	15		Avian and equine strains were considerably more resistant to decay than those derived from human or swine sources	236
Simian virus 40 (SV-40)	21 or 32	15—100	1 hr	The virus was found to be stable at 21°C at all RH levels tested, but aerosols maintained at 32°C were inactivated within 60 min at mid-range RH (50—60%)	237
Foot-and-mouth disease (2 strains)	18—23	10—100	1 hr	Viruses aerosolized from milk and fecal slurry were quite stable at 55% RH; both the strains tested were more stable when aerosolized from milk than from fecal slurry; the virus was found to be inactivated at or below 50% RH, but the RNA retained its infectivity	95
Foot-and-mouth disease (O,BFS,860)	19—22	20—70	1 sec—60 min	The tested strain of FMDV was more unstable when suspended in bovine saliva than cell culture fluid at high RH	97
Newcastle disease virus (3 strains)	20	50—80	4 hr	At least 1% of all virus strains were infectious after 4 hr; no marked differences were observed between strains, but all survived better at 60—80% RH than at 50% RH	206
	Open air conditions			A significant quantity of all 3 virus strains survived at least 30 min	
Swine vesicular disease virus (SVDV-England/72)	19—22	20—80	1 sec—19 hr	SVDV in free aerosols was more stable than FMDV and most stable at and above 55% RH	238
	Open air conditions			Aerosol stable in outdoor conditions on spider microthreads	
EMC	10—37	10—90	1 hr	Aerosolized virus was rapidly inactivated at RH levels below 50%; inactivation of the virus was thought to be due to irreversible changes in the protein coat of the virus resulting from the removal of structurally essential water molecules	82

Table 1 (continued)
EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF AEROSOLIZED VIRUSES

Virus(es)	Temperature (°C)	Relative humidity (%)	Period of aerosol aging	Remarks	Ref.
Feline herpes virus (FHV), feline calicivirus (FCV), infectious bovine rhinotracheitis virus (IBRV), parainfluenza-3 (PI-3), equine arteritis virus (EAV), equine herpes virus type 1 (EHV-1), African swine fever virus (ASFV), vesicular exanthema virus (VEV, type E), vesicular stomatitis virus (VSV), bovine adenovirus type 1 (BAdV-1), equine rhinovirus type 1 (ERV-1)	18—23	20—80	1 hr	ASFV and PI-3 viruses survived well at all RH levels tested when tested only 1 sec after aerosolization; however, after 5 min of storage, both these viruses were found to be sensitive to high RH; the other lipid-containing viruses (EAV, VSV, FHV, EHV-1, IBRV) were also unstable when stored as aerosols in high RH; ERV-1, a picornavirus, was the only virus that survived well at high RH, but poorly on exposure to dry conditions The caliciviruses (VEV, FCV) were sensitive to RH in the 30—70% range; when subjected to aeration, all the lipid-containing viruses which were examined lost infectivity, but nonlipid-containing viruses, including BAdV-1, were stable; the addition of 0.1% peptone reduced losses of virus, and this protective effect was attributed to protection against surface inactivation	72
Influenza (WSNH strain)	21	20—80	1 hr	Minimum virus survival was observed at RH 50—70% with higher recoveries at RH >80% and maximum stability at RH <30%; airborne stability of the virus was found to vary from one preparation of virus to the next for virus propagated in both cell culture and embryonated eggs; polyhydroxy compounds were found to have a protective effect on the airborne stability of the virus	77

Rhinovirus type 2	21—40	40—70	?	In general, virus survived best at high RH	239
Infectious bovine rhinotracheitis (IBR)	6 or 32	30 or 90	3 hr	The virus was found to be more stable when aerosolized from nasal secretions than from MEM; low temperature and high RH were the most favorable for short-term survival	35
Bovine parainfluenza virus type 3 (BPI-3)	6 or 32	30 or 90	1 hr	During aging of aerosols at 32°C and 30% RH, the virus was found to be less stable in Eagle's minimal essential medium (EMEM) than in nasal secretion from a noninfected calf, but at 6°C and 30% RH, the virus was more stable in EMEM; the virus was consistently more stable at 6°C than at 32°C and, at 32°C, the virus was more stable at 30% RH than at 90% RH	36
Bovine adenovirus type 3 (BAdV-3)	6 or 32	30 or 90	3 hr	Virus inactivation was usually more rapid at 30% than at 90% RH, and at 32°C than at 6°C; glucose was found to protect the virus during spraying and amino acids in EMEM were thought to be more protective during aerosol aging	37
Rinderpest	26	20—80	1 hr	The virus was shown to survive well for 30 min at low RH (<40%) and its survival was somewhat reduced at high RH (>80%); virus viability was least at mid-range RH (50—60%)	38
Vesicular exanthema virus	18—23	20—80	1 sec—5 min	Maximum virus survival was seen at high RH; the virus was most sensitive to RH in the range 40—60% in the presence of BSA, glucose, inositol, or phosphate buffer; addition of NaCl stabilized the virus at the medium RH range	87
Japanese B encephalitis virus	24	30—80	1 hr	The half-life of aerosolized virus was 28, 38, and 62 min at RH levels of 80, 55, and 30%, respectively	240

Table 1 (continued)
EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF AEROSOLIZED VIRUSES

Virus(es)	Temperature (°C)	Relative humidity (%)	Period of aerosol aging	Remarks	Ref.
Reovirus type 1 (Lang strain)	21—24	25—95	3 hr	Aerosol stability of infectious and potentially infectious virus particles was maximal at 85—95% RH; an increase in recovery of the aerosolized virus was observed upon prehumidification	80
Calf rotavirus (U.K. strain)	10, 20, 30	20, 50, 90	2 hr	Virus stable at low and high RH; low temperature more conducive to virus survival in air	76
Lassa virus (Josiah strain)	24, 32, 38	30, 55, 80	4—60 min	Biological half-lives of aerosolized virus ranged from 10—55 min; virus survived best at low humidity and low temperature	241
Rotavirus SA-11	20	30, 50, 80	72 hr	The virus was found to survive best at mid-range RH (50%) where its half-life was nearly 40 hr; corresponding half-lives of the virus at low (30%) and high (80%) RH levels were approx. 9 and 2 hr, respectively; in a separate experiment at mid-range RH (50%), 3% of the infectious virus was detectable in the aerosol after 9 days of aging	44
Calf rotavirus, coronavirus	20	30, 50, 80	24 hr	Either rota- or coronavirus was aerosolized in a mixture with polio virus type 1 (Sabin) so that the survival of these 2 viruses could be compared directly with polio virus under the same experimental conditions; both rota- and coronavirus were found to survive best at mid-range RH (50%) with half-lives of >24 hr, and least	41, 42, 242

				well at high RH (80%); on the other hand, polio virus was rapidly inactivated at low (30%) and medium (50%) RH, but had a half-life of about 12 hr at high RH	
Human rotavirus	6, 20	30, 50, 80	24—75 hr	At 20°C, the virus aerosolized from TPB survived best at 50% RH with a half-life of 44 hr; at 30 and 80% RH, its half-life was 24.5 and 3.8 hr, respectively; virus survival was further enhanced at 6°C and mid and low RH. When aerosolized from feces, and held at 20°C and 50% RH, nearly 80% of the virus remained infectious at 24 hr	43
Human coronavirus (229E)	6, 20	30, 50, 80	75 hr	At 20°C, virus half-lives were 67 hr (50% RH), 27 hr (30% RH), and 3 hr (80% RH) when aerosolized from TPB; the lower temperature (6°C) generally enhanced virus survival, but the most dramatic effect was seen at high RH (80%), a 30-fold increase in half-life	42
Human rhinovirus	20	30	24 hr	Infectivity of the virus was rapidly lost at low and medium RH levels; less than 0.25% could be detected in the first air sample; at the high RH level (80%), however, airborne virus had a half-life of 13.7 hr and nearly 30% of infectious virus could be detected even after 24 hr	40

it would not be surprising to find that the decay rates of artificially generated viral aerosols under laboratory conditions would be different from those of natural aerosols of the same virus derived from body secretions or excretions.

Although most of the experimental data on viral aerosols have been generated using artificial spray fluids, some studies have been done where the virus was suspended in and aerosolized from naturally occurring substances such as saliva, milk, and feces. In general, such studies have indicated a protective effect of the natural spray medium. Decay of aerosolized EMC virus sprayed from human saliva was lower at 20 to 40% RH than when the virus was sprayed from either water or balanced salt solution at the same RH level.[82] Elazhary and Derbyshire[35] generated IBR virus aerosols from Eagle's minimal essential medium (MEM) and nasal secretions of calves both with and without antibodies to IBR. It was found that the virus was most stable when it was aerosolized from nasal secretions of a seronegative calf and held at 6°C and 90% RH. Human rotavirus aerosolized from fecal slurry was more stable than the same virus aerosolized from tryptose phosphate broth at 20°C and 50% RH.[54]

The nature of the protecting agent(s) in the natural secretions is unknown, but widely thought to be proteinaceous in nature. Peptone and apolar amino acids were also shown to be protective to airborne EMC and Semliki Forest viruses.[93] The surface protective role of peptone was further substantiated by the work of Donaldson and Ferris[72] using IBR. However, it is also possible that lipid material may play some role in this regard.

In some cases, natural suspending media can be detrimental to the viruses suspended in them. Bovine adenovirus was found to survive better when aerosolized from Eagle's MEM than when the aerosol was generated from nasal secretions of a seronegative calf.[36] In this case, glucose and amino acids in the MEM were considered to protect the virus during spraying and aerosol aging, respectively. Donaldson[94,95] reported that when foot-and-mouth disease virus was aerosolized from milk or fecal slurry, it was more stable than when saliva was used as the spray medium. This difference was first believed to result from the comparatively high pH (8.9 to 9.1) of saliva,[96] but was later shown to be due to a dialyzable component of saliva which was stable to 60°C, but heat labile at 70°C.[97] This limited characterization of the active component suggests the possible involvement of a low molecular weight protein, which could be an enzyme or an enzyme system with a dialyzable cofactor, in virus inactivation.

Knowledge of the mechanism(s) leading to the inactivation of airborne vertebrate viruses is still very rudimentary. Some studies have indicated that the mechanism of virus inactivation may be related more to instabilities in the protein coat of the virus under certain environmental conditions, rather than in the nucleic acid core, which may remain infectious. However, both protein coat and nucleic acid core could be vulnerable to changing environmental conditions or enzyme digestion and the denaturation of either or both of these components may lead to loss of infectivity of the airborne virus. Loss of structurally essential water from aerosolized virions of the picornavirus group may be the mechanism which triggers virus inactivation at low RH levels[82] and allows subsequent denaturation of the surface proteins and/or nucleic acids. In enveloped viruses, on the other hand, low humidities will tend to stabilize the virus envelope and protect the interior of the virion from inactivating agents. Such statements are, however, generalizations, and for any particular virus the degree of hydrophobicity of its surface proteins will also affect its response to RH levels and any other compounds in its immediate surroundings.

A possible example that multiple mechanisms play a role in virus inactivation is Semliki Forest virus.[98] Here, it was observed that removal of salts from the spray fluid resulted in the improved airborne survival of the virus over a wide RH range, whereas

extraneous protein and polyhydroxy compounds added to the spray fluid protected the infectivity of the virus at high and low RH levels, respectively.

No additive has yet been found which is capable of completely stabilizing the infectivity of airborne viruses. However, the presence of certain nonprotein chemicals in the spray or collection medium has also been shown to enhance survival of aerosolized virus. Addition of inositol to the spray fluid has been shown to stabilize a variety of vertebrate viruses. Webb et al.[78] found that the rapid inactivation of aerosolized Rous sarcoma virus at low RH could be prevented by the presence of inositol. A similar stabilizing effect of this chemical was noted for aerosols of influenza, Langat, Semliki Forest, and foot-and-mouth disease viruses.[81,99] On the other hand, inositol was not found to influence the airborne survival of a number of other virus types.[74,87,99] Park[39] compared 10% glycerol and 0.1% bovine serum albumin for their efficacy in stabilizing aerosolized polio virus type 1 (Sabin). Glycerol was found to be superior in this regard, which is consistent with the observation that polio virus is very sensitive to low and medium RH levels. The main mechanism of virus inactivation in this case might, therefore, be expected to be dehydration.

In addition to studying the influence of RH and composition of the spray fluid, Schaffer et al.[77] tested the effects of the propagating host on the airborne stability of influenza A virus. Similar patterns of virus survival were observed when the virus was grown in bovine, chick, or human cells and aerosolized from the cell culture medium. They found no apparent correlation between the airborne stability of the virus and the protein content of the spray fluid above 0.1 mg/mℓ. The presence of polyhydroxy compounds in the spray fluid was also found to give the virus greater airborne stability.

The composition of the aerosol collection fluid is probably determined empirically by each investigator for maximum recovery of the virus under test, but there are no published studies in this regard. Any medium which is not deleterious to the virus can be used, but it is also usual to include an antifoam agent to minimize virus loss.

C. Atmospheric Gases and Aerosolized Chemicals

The presence of certain chemicals in the gaseous state has been shown to inactivate aerosolized viruses. For example, in one of the earliest studies in this area,[100] propylene glycol vapors were found to be effective in preventing infection of mice exposed to experimentally generated influenza virus aerosols. Slobodenyuk and Karpukhin[101] investigated the effect of hydrogen peroxide, chloramine, and hexylresorcine on airborne adenovirus type 3, polio virus type 3, and coxsackie virus type B 1. The minimum concentrations of these compounds needed to bring about a 99.9% reduction of all 3 viruses in 30 min were 10 to 20, 5 to 10, and 5 mg/m^3, respectively. However, much higher concentrations of the same disinfectants were required to bring about a 99.9% reduction in virus titer on a contaminated surface. The basic aim of the experimental work in this area has been to develop the use of these chemicals in the prevention of virus transmission by the airborne route. However, in spite of the immense potential for spray inactivation of aerosolized virus in animal husbandry and infectious diseases hospitals, systematic studies of spray disinfectants are sadly lacking. One interesting approach to inactivation of virus aerosols involves the use of immobilized proteases and nucleases on solid glass or ceramic supports:[102] brief contact with either DNase or crude trypsin was sufficient to inactivate herpes simplex virus in the aerosol phase. The RNA viruses tested, although somewhat sensitive to RNases, were more refractory to protease exposure. Such an approach, possibly in combination with controlled RH levels, could offer prospects for the effective disinfection of recycled air.

Although experimental conditions relevant to indoor environments of artificial light can be relatively easily simulated, airborne virus inactivation under natural outdoor

conditions is much more difficult to evaluate. Hood[103] has designed an indoor system for studying the survival of airborne microorganisms in closed conditions to allow the effect of open air. An alternative means of studying the influence of the open air and daylight on virus survival has been used for foot-and-mouth disease virus.[104] The virus was held as captured aerosol particles on a spider microthread; air and daylight produced no marked effect on virus survival. Such a system has already been used for studying the disinfection of bacterial[105] but not virus aerosols.

Under natural conditions, air contains a large variety of naturally occurring or artifically generated gaseous substances which could affect the survival of viral aerosols. Berendt et al.[106] have shown that a low concentration of sulfur dioxide in air (0.4 ppm) was sufficient to inactivate airborne VEE virus, but concomitant irradiation with simulated sunlight reduced the virucidal properties of the gas. Higher concentrations of sulfur dioxide (3.6 ppm) were able to inactivate the airborne virus even when it was irradiated with artificial sunlight,[107] and this occurred more rapidly at 60% RH than at 30% RH. The rate of inactivation due to a combination of the gas and RH was greater than the sum of the effects produced by these two factors separately. Although the exact mechanism by which gaseous sulfur dioxide inactivates viruses is not known, it may be attributable to the formation of sulfuric acid in moist air. In artificial sunlight, sulfur dioxide may be expected to be photolabile and, therefore, at low levels of sulfur dioxide, the virucidal species may be reduced beyond its effective level. Ehrlich and Miller,[108] also working with VEE, studied the effect of atmospheric nitrogen dioxide on airborne virus inactivation. At 85% RH, atmospheric nitrogen dioxide concentrations of 5 and 10 ppm increased the biological decay rate 3- and 10-fold, respectively. It has been suggested that this virucidal effect of nitrogen dioxide is due to its conversion to nitric or nitrous acid at high RH levels. Apart from these two major pollutants, the effects of the majority of gaseous chemical species in the atmosphere on survival of aerosolized virus are unknown, but it must also be considered that certain of these chemicals may be protective to virus infectivity.

Another factor which deserves consideration in the transmission of airborne viruses is that atmospheric pollutants in nature affect not only the virus, but also the host system present in the same environment. There is ample epidemiological evidence that there is marked involvement of airborne particulates and chemicals in respiratory disease,[109,110] but few specific experimental studies have been conducted and, because of the probable involvement of host factors in virus disease transmission, this topic is considered beyond the scope of this review.

D. Irradiation

Following the early studies of Wells and Brown[111] and Wells and Henle,[112] Edward et al.[113] observed the virucidal effects of ultraviolet (UV) rays on certain types of airborne viruses. Their experiments demonstrated that aerosolized influenza and vaccinia viruses could be rapidly inactivated when irradiated with light of a wavelength 2537 Å. Similar virus inactivation was observed for Rous sarcoma virus irradiated at 2800 to 3200 Å.[32] Similar findings were reported for coxsackie B1, Sindbis, influenza A, and vaccinia viruses.[114] In the latter case, air containing these viruses was passed through the UV cell at 100 ft^3 per min, but even this brief exposure resulted in more than 99.9% virus inactivation. Adenovirus type 2 was shown to be somewhat more resistant to UV inactivation under the same conditions;[114] only 96.8% of the virus was inactivated.

Berendt and Dorsey[115] reported that simulated solar radiation is deleterious to aerosols of VEE virus. The lethal effect of such radiation could be enhanced by the presence of sulfur dioxide at high RH levels.[106,107] Attempts have been made to use such lethal effects of UV to interrupt virus transmission by the airborne route. UV disinfec-

tion of airborne influenza virus has been shown to be effective in preventing infection of mice exposed to experimentally generated aerosols of the virus.[100] Riley[116] has also suggested that, under appropriate conditions, airborne transmission of influenza virus could be interrupted by UV disinfection of air. Application of UV irradiation to the control of measles outbreaks in schools has also been reported[117,118] and it appeared to be effective.

It would therefore appear that UV irradiation may be a useful means to interrupt spread of an aerosolized virus under controlled conditions. There is evidence that daylight and room lighting are deleterious to certain viruses.[119] However, there is usually little low wavelength UV present in normal sunlight or room lighting, and the very fact that virus transmission can occur under these conditions suggests that the longer UV wavelengths may have comparatively little effect on the survival of some aerosolized virus. Furthermore, diurnal cycles of light and dark ensure that there are usually periods when aerosolized virus would not be exposed to any UV radiation. Foot-and-mouth disease virus is known to travel many kilometers in the air, and such dispersion may be particularly favored at night.[120] Therefore, under natural conditions it is not known what role UV irradiation would play in preventing transmission of aerosolized viruses.

VI. VIRAL AEROSOLS GENERATED IN THE WORK AND HOME ENVIRONMENT

The first record of a laboratory-acquired infection dates back to 1885.[121] Since then, much attention has been given to the possible spread of viruses and other infectious agents to laboratory workers involved in their handling. Sulkin and Pike[122] summarized the data from 222 reports of laboratory-acquired infections. The following types of viruses were among the infectious agents involved: eastern, western, and Venezuelan equine encephalitis; Russian spring summer encephalitis; louping ill; lymphocytic choriomeningitis; poliomyelitis; encephalomyocarditis; Newcastle disease; yellow fever; dengue fever; Rift Valley fever; Colorado tick fever; mumps; influenza; hepatitis; rubella; and agents of viral diarrhea. Analysis of the reports indicated that 30% of the infections were due to contaminated laboratory air.

During the past three decades, numerous studies have been conducted on the production of viral aerosols by several procedures commonly used in laboratories or hospital settings. Potentially dangerous aerosols from virus-containing material were found to be generated during centrifugation,[123-128] homogenization,[129,130] use of autoanalyzer equipment,[126] opening of screw-capped containers,[131] recovery of glass ampules from liquid nitrogen storage,[132] and operation of hemodialysis units.[133,134] Operation of air-turbine dental handpieces[135,136] and use of ultrasonic devices for cleaning surgical instruments[137] have also been found to be capable of generating viral aerosols.

Pike[138] reviewed the published reports on microbiology laboratory procedures and accidents which could result in the generation of viable airborne particles. He concluded that 27% of the cases of laboratory-acquired infections were due to airborne viruses. It was further noted that cases in research laboratory personnel accounted for more than 67% of the laboratory-related infections.

In view of the above, several investigators have emphasized the importance of proper facilities and training for the safe handling of potentially infectious materials.[133,138-147] Recent improvements in the design and construction of biohazard containment equipment and better enforcement of biosafety procedures may have reduced the hazards of some laboratory procedures in many parts of the world. However, health risks involved in handling potentially dangerous clinical specimens and in performing some

laboratory procedures, such as centrifugation, which cannot usually be conducted in standard containment equipment, emphasize the vigilance which must be exercised to prevent airborne viral transmission in the laboratory setting.

Baylor and Baylor[148] have shown that viruses tend to accumulate at the liquid-air interface. Aeration of a virus-containing liquid resulting in the bursting of bubbles, therefore, can lead to the ejection of virus particles into the air; the concentration of virus particles per unit volume of the ejected liquid has been found to be many times higher than that of the source water itself. More recently, Blanchard and Syzdek[149] have reported that most particles generated as a result of the bursting of bubbles are in the 1 to 10 μm range with potential for retention in the lungs on inhalation. These findings have further enhanced the interest in the possiblity of generation of biohazardous viral aerosols during the handling of infectious materials as well as from wastewater treatment and disposal practices.

Raw and treated sewage and the sludge from sewage treatment processes usually contain large numbers of vertebrate viruses.[150] The use of such wastes for spray irrigation is believed to result in the generation of infectious viral aerosols.[151] Enteric viruses have been recovered up to 50 m or more downwind from irrigation sprinklers.[152-154] The dumping of sewage sludge into the oceans may also lead to generation of viral aerosols in the surf[155] which may be carried inland under suitable environmental conditions. There is no direct evidence for the transmission of human or animal viral disease by spray irrigation, but it has been suggested[156] that communities surrounding such irrigation sites may be at a greater risk of exposure to communicable diseases. Donaldson and Ferris[157] have suggested that spray irrigation of wastes contaminated with swine vesicular disease virus may result in widespread dissemination of infectious virus to the environment, but they are less certain whether the disease is spread directly by the airborne route.

The possibility of disease transmission due to aerosols generated by flush toilets has received only limited attention. This is in spite of the belief that, apart from sneezing and coughing, flushing of toilets must be the most common process in the production of infectious aerosols of human enteric pathogenic viruses.[158] Gerba et al.[159] investigated the role of household toilets in the production of viral (polio virus type 1) aerosols. After the initial contamination of the toilet bowl water with the agent, it was found to be aerosolized by several subsequent flushes. This was due to virus adsorption to the porcelain surface of the toilet bowl with gradual elution occurring after every flush. Furthermore, aerosols generated by the flushing of the toilet resulted in the spread of the viral contamination to the air as well as to other surfaces in the washroom. Such aerosol spread of viruses from flush toilets could be important both in the home and in public facilities. Wallis et al.[160] were able to regularly detect the presence of polio virus in aerosols generated by the flushing of toilets containing virus-contaminated feces. Diaper changing in infants and young children could also be an important source of enteric virus aerosols and any infectious virus materials which settle to the surface may be subsequently resuspended in the air by routine cleaning processes.

The potential for spread of disease by infectious virus aerosols may be greatly increased where susceptible individuals are crowded together and air circulation is poor or air is recycled. Such situations may occur in institutional settings such as hospitals[161] or schools,[162] on public transportation vehicles,[163] or in animal husbandry facilities.[164,165]

VII. CHALLENGE OF HUMAN VOLUNTEERS AND EXPERIMENTAL ANIMALS TO ARTIFICIALLY GENERATED VIRAL AEROSOLS

Several studies have experimentally challenged susceptible human or animal hosts

with artificially generated viral aerosols: information from published papers on this is summarized in Table 2. In most of these studies, susceptible hosts could be infected upon exposure to such experimentally produced aerosols. However, the level of virus challenge was often unknown and, in some cases, may have been unrealistically high. Furthermore, in spite of the importance of RH in the survival of airborne virus, many of the studies quoted failed to mention the RH levels at which the challenge studies were conducted. Many of the studies on aerosol challenge of susceptible hosts have employed virus strains adapted to growing in cell cultures or embryonated eggs, and it is not known how such adaptation to the laboratory environment may affect the airborne survival and infectivity of these agents. In most cases, there are obvious practical difficulties in obtaining sufficient quantities of body fluids with suitably high titers of infectious virus. Even if such material could be obtained, accurate quantitation of its infectious virus content may present problems. Therefore, from the findings of these laboratory-based studies, it cannot be extrapolated automatically that airborne spread of these viruses regularly occurs in nature. Variations in the experimental design of these investigations, and the inherent differences in the viruses and hosts used, make it very difficult to carry out any direct comparisons of the data generated. However, the following general conclusions could be drawn from the findings of these studies:

1. Inhalation of the viral aerosols often results in the initial replication of the virus in the respiratory tract.
2. Compared to other means of virus inoculation, aerosol challenge may require smaller numbers of virus infective units to infect a susceptible host.[166]
3. Virus infection through the aerosol route usually results in the production of signs and symptoms typical of the disease.[167]
4. The simultaneous aerosol challenge of a host with a bacterial and a viral pathogen can produce a synergistic response.[168]
5. The presence of virus in the respiratory secretions of the infected hosts makes them a source of viral aerosols.
6. Inhalation of aerosols of attenuated viruses leads to the seroconversion of susceptible hosts.

VIII. RECOVERY OF NATURALLY OCCURRING VIRAL AEROSOLS

Due to a general lack of suitable methodology, a very limited number of attempts have been made to recover infectious viruses present in naturally occurring aerosols. The study by Artenstein and Miller[169] was among the first ones to use a large-volume air sampler (LVAS) for the recovery of naturally occurring virus from the air. LVAS have also been successfully used for the recovery of naturally occurring aerosols of coxsackie virus A-21,[4,170] rabies virus,[170,171] adenovirus type 4,[170] rabbit pox and smallpox viruses,[56,172] and polyomavirus.[173] The high cost and relative inefficiency of such devices have kept their use very limited.

In a field study aimed at recovering aerosols containing rabies virus, the LVAS was found to be somewhat superior to an AGI.[4] This may have been due to the capacity of the LVAS to sample much larger volumes of air and concentrate the particulate matter in relatively small amounts of collecting fluid. In general, the LVAS can yield a 100-fold greater concentration of particulate matter than the AGI.[171]

Certain LVAS studies of natural aerosols in outdoor air at a wastewater treatment facility in the U.S.[174] or at a wastewater irrigation site[153] have failed to demonstrate the presence of indigenous vertebrate enteric viruses. On the contrary, Teltsch and Katznelson[152] could detect echovirus type 7 in the air 40 m downwind from an effluent

Table 2
EXPERIMENTAL CHALLENGE OF ANIMALS OR HUMAN VOLUNTEERS TO VIRAL AEROSOLS

Virus type(s)	Host(s)	Remarks	Ref.
Influenza and ectromelia	Mice	The infection resulting from inhalation of virus is described; they suggested that this method may be used with advantage in studying in detail the history of lung lesions and obtaining more uniform infection of large batches of mice; it was deduced that only 1% of virus may reach the lungs of mice breathing normally in an atmosphere containing dispersed droplet nuclei of influenza virus	243
Influenza A (PR 8 strain)	Mice	Exposure of mice to influenza virus aerosol produced 100% mortality at 30 and 80% RH compared to only 22.5% at 50% RH	244
Influenza A (PR 8 strain)	Mice	Studied the pathogenesis and pathology of airborne influenza A infection from its earliest inception through recovery	245
Influenza A (W.S. strain)	Mice	The virus was found to have greater infectivity when introduced by the airborne route rather than by instillation in the nose	227
Influenza A (PR 8 strain)	Mice	Sublethal doses of the virus and *Diplococcus pneumoniae* (type 1) were found to be capable of interacting in the pathogenesis of pulmonary disease in the mice; synergistic interaction was evidenced by the high mortality in the combined infection groups	168
Monkey B virus	Rabbits, monkeys, guinea pigs, rats, mice	Among 5 species of animals exposed to monkey B virus, rabbits were found to be the most susceptible	246
Vaccinia, rabbit pox, cow pox, monkey pox, and variola	Cynomolgus monkey	Aerosolized pox viruses were found to be infective for cynomolgus monkey; the pattern of the disease consisted of a febrile reaction, distinctive constitutional signs of illness, variable mortalities, and an immunological response in the form of neutralizing antibody	247
Tick-borne encephalitis virus (B3 strain)	Monkey	Infection of cynomolgus monkeys with tick-borne encephalitis virus by exposure to aerosols caused no clinical signs of involvement of the central nervous system; active immunization of monkeys with a vaccine from tissue culture protected the animals reliably against even relatively large amounts of nebulized tick-borne encephalitis virus	248
Venezuelan equine encephalitis (attenuated)	Guinea pigs, mice, monkeys	Respiratory exposure to living attenuated virus is suggested as an effective method of active immunization	249
Tick-borne encephalitis virus	Mice	Approximate inhalation lethal dose of viral aerosol was found to be equivalent to 10—40 intracerebral LD_{50}; the incubation period following aerosol infection was similar to that following intranasal infection; the advantages of active immunization compared with the administration of specific gamma-globulin were also indicated	250
Coxsackie A21, adenovirus types 26 and 27	Human volunteers	A method for producing a standard infection in volunteers by use of an aerosol chamber was established	251

Table 2 (continued)
EXPERIMENTAL CHALLENGE OF ANIMALS OR HUMAN VOLUNTEERS TO VIRAL AEROSOLS

Virus type(s)	Host(s)	Remarks	Ref.
Yellow fever and Rift Valley fever viruses	Rhesus monkey, hamster	Rhesus monkey was found to be highly sensitive to aerosol of yellow fever virus; one LD_{50} was equivalent to <1/6 of mouse intracerebral LD_{50}; both hamsters and monkeys were exposed to Rift Valley fever virus aerosols; an LD_{50} for hamsters of 0.5 mouse intraperitoneal (MIP) LD_{50} was established; monkeys were exposed to inhaled doses as low as 76 $MIPLD_{50}$, and all developed viremias with subsequent positive serology	79
Avian lymphomatosis virus	Chicks	Transmission of the lymphomatosis agent via the aerial route was established by interconnecting ventilation systems from modified Horsefall units containing infected chicks to ones containing susceptible ones; the clinical and pathological manifestations of the disease were found to be indistinguishable from those of parenterally induced or natural infections	211
Influenza A1 and A2	Mice	Infector mice were found to transmit influenza virus infection most readily during the period 24—48 hr after initiation of their infection; the mouse-adapted strain of A2 virus was found to be more readily transmitted than the CAM strain of influenza A1 virus, although the CAM strain induced higher pulmonary virus titers and more extensive lung lesions	252
Mouse hepatitis virus	Weanling Namru mice	Approximately 80% of the young mice developed infection when exposed to the virus aerosol	253
Hog cholera	Pigs	Pigs immunized with hog cholera vaccine by intramuscular (IM) injection were challenged with virulent virus by either aerosol or IM route; both aerosol and IM challenges yielded similar results	59
Coxsackie A21	Human volunteers	Virus transmission from an infected volunteer to a noninfected partner, living in the same flat, was shown to occur in 3 out of 20 tests; infection was not transmitted when volunteers either mixed for a few hours with infected subjects or inhaled air into which they had just sneezed	254
Rhinovirus NIH 1734	Human volunteers	Aerosol exposure to the virus resulted in infection and illness in each of 8 antibody-free volunteers	255
Foot-and-mouth disease (FMD)	Cattle	Cattle were found to be infected with experimentally aerosolized FMD virus; air in loose boxes containing experimentally infected cattle was sampled by "Porton-type" impingers; virus collected from the air was concentrated by adsorption and was detected by IP inoculation into unweaned mice	198
West Nile virus	Mice	Virus dissemination in selected mouse tissues was studied after exposure to an aerosol of West Nile virus; maximal multiplication of the virus was observed in CNS, but the first sign of the presence of virus appeared in the olfactory bulbs; this occurred before its appearance in the mid-brain and cerebellum; invasion of the CNS by this virus was presumed to occur through the olfactory pathway	256
Encephalomyocarditis Columbia-SK, mengo, ME virus	Mice	Response in mice varied according to strains; virulence correlated with plaque size; small plaque-forming strains of ME and mengo were essentially avirulaent; response of mice to infectious RNA of all these viruses was the same	257

Table 2 (continued)
EXPERIMENTAL CHALLENGE OF ANIMALS OR HUMAN VOLUNTEERS TO VIRAL AEROSOLS

Virus type(s)	Host(s)	Remarks	Ref.
Influenza A2	Human volunteers	Viral aerosol-containing air (10 *l*) was inhaled by test subjects; the human infectious dose of the virus for seronegative subjects was found to be approximately 3 tissue culture infective dose $(TCID)_{50}$	258
Coxsackie A21, rhinovirus (NIH-1734), adenovirus type 4	Human volunteers	Volunteers were inoculated with these viruses by means of nasal instillations or inhalation of aerosols; it was concluded that airborne transmission could account for some naturally occurring acute respiratory diseases; this was further confirmed by the production of airborne virus during coughs and sneezes	259
Adenovirus type 4 (75680)	Human volunteers (military recruits)	Volunteers who possessed serum antibody prior to viral exposure were protected, but the remaining subjects developed illness indistinguishable from the naturally occurring illness	260
Venezuelan equine encephalomyelitis	Pigeons, leghorn chickens, Peking ducks	Although marked species differences occur, it was concluded that the virus can infect avian hosts through the lower respiratory tract; the minimal infective dose for white Carneau pigeons was found to be between 135 and 374 $MICLD_{50}$ inhaled in not more than 1 min	261
Vaccinia, rabbit pox, variola	Rabbits, rhesus monkeys	All 3 viruses proved highly infectious to animals by the respiratory route and multiplied at similar sites in the lungs; rabbit pox virus, thought to have arisen from oculonasal discharges, was also sampled from the air	262
Newcastle disease virus (NDV)	Chickens	Chickens vaccinated by aerosols were found to be resistant to both IM and aerosol challenges, whereas those vaccinated IM were not protected from aerosol challenge	263
Parainfluenza 1 (Sendai)	Mice	Pathogenesis and pathology of the disease were found to be influenced by virus dose; the speed and magnitude of the antibody response also correlated positively with the amount of virus administered	264
Yaba virus	Monkeys	It was concluded that aerosolized Yaba virus is potentially hazardous to animal care and laboratory workers	265
Coxsackie A21	Human volunteers	Subjects given small particle aerosols (0.3—2.5 μm diameter) showed significantly greater antibody rise, irrespective of clinical response, than when the agent was given as large-particle aerosols (15 μm diameter)	266
Rauscher murine leukemia virus	Mice	First investigation describing the aerosol stability of the virus and its ability to spread by the airborne route	267
Rauscher murine leukemia virus (RMLV)	Mice	39.5% of RMLV-exposed mice developed leukemia within 25 months of exposure to RMLV aerosols	190
Yaba, Rauscher murine leukemia, adenovirus type 12, Rous sarcoma virus	Monkeys	Showed that tumor viruses could be readily transmitted to susceptible hosts via the aerosol route, and emphasized the potential hazards due to these agents in animal colonies and laboratories	268
Adenovirus type 12	Newborn Syrian hamsters	Airborne virus was shown to be pathogenic for newborn Syrian hamsters	234
Parainfluenza type 1 (Sendai)	Mice	Greater rates of transmission were observed at higher RH; transmissibility did not increase after serial airborne passage of the virus	269
Adenovirus 4, coxsackie A21	Human volunteers	Determined infectivity of these viruses by aerosols	270

Table 2 (continued)
EXPERIMENTAL CHALLENGE OF ANIMALS OR HUMAN VOLUNTEERS TO VIRAL AEROSOLS

Virus type(s)	Host(s)	Remarks	Ref.
Marek's disease virus (MDV)	Chickens	Exposure to effluent air from "donor cages" housing infected animals resulted in a high incidence of MDV infection in test chicks; passage of contaminated air through certain filters partially or completely prevented such infection	164
Newcastle disease virus	Chickens	Unvaccinated birds shed much higher levels of virus than those previously vaccinated	206
Vesicular stomatitis	Mice	Exposure of mice for 1 hr to ozone resulted in a 70% increase in respiratory deposition of the virus	271
Influenza A (PR 8 strain)	Mice	Under conditions of aerosol inhalation, mice were found to be a suitable model for studies on pathogenesis	272
Influenza A (PR 8 strain)	Mice	Concluded that the finding of extrapulmonary virus was in direct quantitative relationship to the extent of lung involvement	273
Influenza	Mice	The resistance of mice to viral pneumonia was affected by the presence of manganese dioxide	225
Bovine rhinotracheitis	Cattle	Compared clinical and immunological responses after infection with viral aerosols or IM inoculation; in both cases, the virus generally elicited comparable levels of serum antibody but not measurable nasal antibody; moreover, aerosol-exposed cattle shed virus in their nasal passages while the others did not	274
Feline caliciviruses	Cats	Showed that aerosol transmission probably plays little part in the epidemiology of infections by these viruses	275
African swine fever (KWH/12)	Pigs	Uninfected animals (recipients) were held for 6 days on a platform 2.3 m above 8 infected pigs (donors); the distribution of the virus titer in selected tissues of 10 recipients was determined at 2-day intervals over the following 8 days; virus was not detected in any of the tissues obtained from 2 pigs killed at 0 and 6 days after exposure and pigs had developed generalized infection between 2 and 8 days after exposure; the titer of the virus in the lymph nodes draining the lower respiratory tract of 3 pigs was considerably greater than that in the nodes draining the upper respiratory tract; it was, therefore, concluded that the primary route of infection in these pigs was through the lower respiratory tract	276
African swine fever (KWH/12)	Pigs	Demonstrated infection of experimental animals after challenge with aerosolized virus	277
Rinderpest	Cattle	Tested 4 different strains of the virus and confirmed the earlier reports of the infectivity of airborne rinderpest virus for cattle; conditions of low or high relative humidity were shown to increase the probability of disease transmission by the respiratory route, but any aerial spread across distances greater than a few meters was believed to occur principally at night	38
Feline caliciviruses	Cats	Compared large (ep) and small (mp) plaque-forming strains by aerosols or direct intranasal instillation; by both routes of inoculation, the disease produced by the mp strain was clinically and pathologically less severe than that produced by the ep strain	278
Influenza	Mice	Mice exposed to virus aerosols at 50% RH and 22°C became infected and viral antigen was detected in cells of the respiratory tract	279

Table 2 (continued)
EXPERIMENTAL CHALLENGE OF ANIMALS OR HUMAN VOLUNTEERS TO VIRAL AEROSOLS

Virus type(s)	Host(s)	Remarks	Ref.
Bovine respiratory syncytial virus (Quebec strain)	Holstein calves	Animals exposed to aerosols of the virus manifested moderate to severe signs of respiratory disease; virological and serological assessment demonstrated that the observed clinical picture was due to infection with this virus	167
Japanese B encephalitis	Mice, rats, hamsters, guinea pigs, squirrel monkeys	Mice and hamsters were highly susceptible to aerosol challenge; guinea pigs and rats seroconverted but survived the infection; squirrel monkeys only died after a high dose of infectious virus	240
Rauscher murine leukemia	Mice	Showed that the infection of healthy BALB/c mice with leukemogenic virus through the upper respiratory tract (intranasal, aerosol), or through a skin lesion, was possible	280
Parainfluenza-3	Calves	This is the first report of extensive purulent pneumonia in calves after exposure to aerosols of the virus and *Pasteurella haemolytica*, which were found to act synergistically	281
Pseudorabies	Pigs	Virus was transmitted to seronegative pigs which were exposed to air from loose boxes containing infected pigs	209
Infectious bronchitis virus (Australian T strain)	Chickens	The pathogenesis of this virus in 4-week-old chickens was investigated by administering the virus by different routes; birds infected by the aerosol route had earlier and slightly more severe respiratory symptoms; on the other hand, when the same experiments were done on 18-day-old chickens housed in a cold environment, it produced more severe symptoms compared to those receiving supplementary heat	282
Bovine herpes virus 1 (BHV-1) and *Pasteurella hemolytica*	Calves	The dose-response relationship was examined between BHV-1 and disease in calves exposed to a constant level of *P. haemolytica;* the 50% effective dose for fibrinous pneumonia under these experimental conditions was approx. 10,000 infectious units inhaled per calf	283
Lassa fever	Monkeys	Monkeys exposed to a small particle virus aerosol, containing 465 pfu or greater, became infected and died; the median infective dose for guinea pigs was 15 pfu	241
Mouse rotavirus	Mice	Neonatal mice developed acute gastroenteritis within 48 hr of exposure to virus aerosols	23

irrigation site in Israel; a large-volume liquid scrubber (Aerojet-General) was used in this study, and the virus was recovered in 4 out of 12 runs. Several other airborne enteroviruses have been detected up to 100 m downwind from similar wastewater irrigation sprinklers.[154] It has been estimated[154] that, due to sampling limitations, virus recovered from environmental air samples may be one to two orders of magnitude less than the actual numbers of infectious virus present in the air sampled.

Table 3 summarizes additional details from published studies dealing with the sampling and detection of viruses in naturally occurring aerosols. As was the case for the experimental challenge of susceptible hosts (Table 2), many of the studies quoted here fail to mention the RH levels at which the sampling was conducted. An RH level deleterious to virus survival could readily influence the outcome of a recovery experiment, especially when small numbers of virus are involved.

White et al.[175] developed a relatively simple LVAS. It is not yet commercially available. A prototype of this sampler is available in our laboratory and has been tested for

Table 3
SAMPLING AND DETECTION OF NATURALLY OCCURRING VIRAL AEROSOLS

Locale of air sampled	Sampler used	Volume of air tested	Virus recovered	Remarks	Ref.
Infectious diseases hospital	Glass sampler containing tightly packed cotton	10 *l*/min for 2—60 min	Variola	Virus recovered in only 1 of 38 trials, but sampling equipment was fairly primitive	284
Army hospital room of 1440 ft^3	Large-volume air sampler (LVAS), Litton Systems, Inc.	1785 ft^3 in 5 min	Adenovirus type 4	Air was collected in 180 m*l* of sampling fluid and 1 $TCID_{50}$ of the adenovirus per 277 ft^3 of air was recovered	169
Room occupied by virus-infected human volunteers	LVAS	82% of total room air in 12 min (120,000 *l*)	Coxsackie A-21	Data obtained strongly suggest that an infected person may discharge sufficient virus into the air to account for the airborne transmission of this disease	4
Rooms containing infected rabbits	Electrostatic precipitator or glass impinger	?	Rabbit pox	Low levels of virus were recovered with the electrostatic precipitator, but not with the glass impinger; this was presumed to be due to the relative sample sizes obtained by the two methods of aerosol collection	262
Air inside bat-infested caves	LVAS or all-glass impinger (AGI)-4	100,000—300,000 *l*	Rabies	First report on the isolation of rabies virus from the air; found the LVAS superior to the AGI-4 in virus recovery	171
Air from loose boxes housing infected animals	LVAS and multistage liquid impinger	60,000 *l*	Foot-and-mouth disease (FMDV)	More airborne virus was detected from infected pigs than from cattle or sheep; under appropriate environmental conditions, it was estimated that virus could travel up to 100 km	285
Laboratory animal room housing infected rabbits	Slit and Anderson sampler using adhesive sampling technique	60 ft^3 in 1 hr	Rabbit pox	The higher rate of virus recovery compared with a previous report was attributed to large volume of air sampled and samples assayed immediately	172
Poultry house containing infected chickens	LVAS and multistage liquid impinger	200 and 33,000 *l*	Newcastle disease	Comparable amounts of virus were recovered with the 2 samplers: about 320 egg lethal dose $(ELD)_{50}$ from 200 *l* and about 500,000 ELD_{50} from 33,000 *l* of air; moreover, viable virus was detected in open air 64 m downwind from the virus-contaminated premises	206
Animal house containing infected mice	LVAS and AGI-4	18 m^3	Polyoma	Samples obtained by LVAS were further concentrated by high-speed centrifugation; airborne virus was detected in 4 out of 6 samples	173

Table 3 (continued)
SAMPLING AND DETECTION OF NATURALLY OCCURRING VIRAL AEROSOLS

Locale of air sampled	Sampler used	Volume of air tested	Virus recovered	Remarks	Ref.
Operational wastewater spray irrigation site (Israel)	Large-volume Aerojet-General liquid scrubber	600 *l*/min for 15—20 min	Echovirus-7	More virus was detected during nighttime sampling compared with daytime sampling; the virus was isolated in 4 of 12 samples collected 40 m downwind from the sprinkler	152
	Cyclone scrubber LVAS	600 *l*/min for 2 hr	Echovirus types 1, 25, and 29, polio virus type 2, coxsackie B1	Enteroviruses were recovered up to 100 m from the irrigation sprinklers with a mean concentration in air of 0.04 isolates/m³ of air sampled; the RH level during sampling (40%) was not the optimum usually observed for enterovirus survival in air	154
Operational wastewater spray irrigation site (Texas)	LVAS	1000 *l*/min for 30 min	Polio virus-1 and coxsackie B3	Described a procedure to determine low levels of viruses aerosolized from wastewater spray irrigation	153
Animal holding facilities	LVAS	60,000 *l*	Pseudorabies	Virus was regularly detected in air samples taken from boxes housing infected pigs	209

the recovery of respiratory and enteric viruses from artificially generated aerosols.[39] Experiments are now underway to test its feasibility in the detection of such viruses in naturally occurring aerosols in hospitals and at waste treatment plants.

IX. SPREAD OF NATURALLY OCCURRING VIRAL INFECTIONS BY THE AIRBORNE ROUTE

Several reports have been published on the airborne spread of naturally occurring viral infections in both humans and animals. In some of these cases, aerosol transmission may be the chief mode of virus transfer from infected to susceptible hosts (e.g., measles virus, influenza). Other documented reports may represent isolated instances of airborne transmission of a virus which is normally spread by direct contact or carried by other vehicles such as water, food, and fomites. In either case, it must be emphasized that all viruses which can survive a particular aerosolization process have the potential for airborne transmission. It is, however, interesting to note that certain viral infections of the respiratory tract (e.g., the common cold) that were thought to spread mainly by the airborne route are now being considered to have direct contact followed by self inoculation as the principal means of their transmission.[176]

Details are given below of a number of documented incidences of airborne transmission of viral infections.

A. Smallpox

Although several reports of the airborne transmission of smallpox are available,[177,178] the outbreak described by Wehrle et al.[161] is perhaps the most dramatic and

best documented. Prior to this report, there seemed to be some doubt about the role of air in the spread of this disease.[179] The outbreak, which occurred in the Federal Republic of Germany, involved a total of 17 persons. It was suggested that the low level of relative humidity in the hospital air appeared to have helped virus survival, and air currents led to its rapid dissemination within the hospital environment. However, experimental work with members of the pox virus group has suggested that RH may have little effect on their airborne survival.[78,180]

Another attempt to recover smallpox virus from air adjacent to infected patients in a smallpox hospital[181] yielded less aerosolized virus than the investigators anticipated. No comments were made on the RH levels in the hospital. Smallpox contamination of bed linen[182] could also resuspend the virus in the air during housekeeping procedures.

The human case of smallpox at the medical school, University of Birmingham, England, is believed to have acquired the virus when it was carried by air from a research laboratory on the floor below.[182]

B. Influenza

Moser et al.[163] have described an influenza outbreak where the air inside an airliner was found to be the vehicle for the spread of the virus. The jetliner, with 54 persons on board, had a 3-hr delay in takeoff due to some mechanical defect. Most of the passengers, including one person with the clinical symptoms of influenza, remained in the aircraft. The ventilation system within the aircraft was inoperative during this delay. Within 72 hr of this incident, 72% of the passengers became ill with headache, sore throat, fever, fatigue, and myalgia. A virus antigenically similar to influenza A/Texas/1/77 was recovered from 8 of 31 passengers tested, and 20 of 22 ill passengers had serologic evidence of infection with this virus.

C. Measles

Riley et al.[162] have recorded an outbreak of measles in a modern suburban elementary school in upstate New York. The index case was a girl in the second grade. This outbreak, which occurred in the spring of 1974 and resulted in 28 cases in 14 different classrooms, was notable for its explosive nature. Also of interest was the fact that 97% of the children in this school had been vaccinated against measles, most of them having received the vaccine when they were less than 1 year old. Analyses of the data from this outbreak have provided a basis for apportioning the chance of airborne infection in classrooms and from exposure in school buses.

Centers for Disease Control[183] in the U.S. have reported an airborne outbreak of measles which began with an international importation in a 7-month-old baby who arrived in the U.S. from Korea for adoption. She infected four other children in a pediatrician's office; two additional measles cases occurred subsequently in family members of these four children. Although the exact mode of transmission in this instance could not be proved, transmission via fomites seems less likely than airborne transmission because measles virus is believed to survive for only a short time on dry surfaces.

D. Chicken Pox

Leclair et al.[184] have described an outbreak of chicken pox occurring in a pediatric hospital. The virus is believed to have been spread by the airborne route. Recently, Gustafson et al.[185] reported another nosocomial outbreak of chicken pox. It occurred in Nashville, Tennessee, in November, 1980, and involved eight patients. Although the index patient remained in strict room isolation during his hospital stay, the virus is believed to have escaped to the surrounding area. Subsequent airflow studies showed

that the index patient's room was under positive pressure with respect to the corridor. On the basis of their observations, the authors felt that airborne transmission of varicella was a common mode of spread in hospitals.

E. Lymphocytic Choriomeningitis

An outbreak of lymphocytic choriomeningitis (LCM) occurred in 1972 to 1973 in personnel at a medical center in Rochester, New York.[186] Epidemiological and virological studies indicated that the source of the infection were Syrian hamsters being used there for tumor research. Cell cultures derived from these animals were also found to be contaminated with the virus. The cases of human infection were shown to occur not only through direct contact with the animals, but also from mere presence in the room where the animals were being held.

F. Epstein-Barr

Spread of Epstein-Barr (EB) virus to 5 out of 18 technologists in a clinical laboratory has been attributed to the airborne route.[187] Other possible airborne outbreaks of EB virus have been reported.[188,189]

G. Rauscher Murine Leukemia Virus

Rauscher murine leukemia was experimentally transmitted to BALB/c mice by exposing them to aerosols of the virus.[190] Mice in contact with aerosol-exposed cagemates also developed the disease.

H. Rabies

Two investigators who had spent some time inside bat-infested caves in Texas subsequently died of rabies.[191] Exposure of these persons to the virus by the airborne route was strongly indicated. That the atmosphere of the caves did indeed contain airborne rabies virus was later proved both by exposing a variety of rabies-susceptible animals (in insect-proof cages) to the air inside these caves and by direct isolation of the virus from the air.[171] Airborne spread of this virus is also believed to have been responsible for an unusual outbreak of rabies in a wild carnivore colony at a research station in New Mexico.[192]

A research veterinarian working on an experimental rabies vaccine contracted the disease and died.[130] Exposure to aerosols of the virus generated in a homogenizer was the most likely mode of spread in this case. That the virus entered the body of this individual through the process of inhalation was further confirmed when rabies virus particles were detected in the myelinated nerve fibers of his olfactory glomeruli.[193] Tillotson et al.[129] have reported another case of rabies in a laboratory technician who was accidentally exposed to an aerosol of modified live rabies virus vaccine.

I. Rotaviruses

Kraft[194] believed that epizootic diarrhea of infant mice (EDIM) was being transmitted by air in her mouse colony. In order to overcome this problem, she devised cages with air filters and conducted the handling and inoculation of the animals under a special hood with air locks and negative pressure. These precautions were apparently very helpful in stopping the spread of the infection from virus-inoculated to control animals.[165] We have found this mouse rotavirus to survive well in the airborne state.[54] A recent study[23] was able to produce diarrhea in infant mice by challenge with rotavirus aerosols. Middleton et al.[195] reported the spread of human rotavirus infection from inoculated to noninoculated animals housed in separate rooms in the same animal care facility. They, however, did not indicate if they considered this cross-infection to be due to the aerial spread of the virus.

The 1964 outbreak of acute gastroenteritis in a group of islands in the mid-Pacific was shown to be due to a rotavirus with the help of retrospective serological studies.[196] The high attack rate and the rapid spread of the outbreak were strikingly similar to those of influenza. On these grounds, it is suggested that air may have acted as a vehicle in the spread of this outbreak.

J. West Nile Virus

Nir[197] has reported a case of this infection in a laboratory worker and circumstantial evidence pointed to an airborne spread of the virus. This possibility was subsequently substantiated by the successful challenge of experimental animals with West Nile virus aerosols.

K. Hantaan Virus (Korean Hemorrhagic Fever)

Lee and Johnson[198] have recorded nine clinically apparent cases of Hantaan virus infection at the Korean University Virus Institute (Seoul) which occurred there between 1971 and 1979. All of these were directly related to trapping of wild rodents or work with naturally or experimentally infected animals. These cases were acquired in the months of November to April and none of them was associated with accidental parenteral inoculations. The facts strongly indicate aerial spread of the virus through aerosols generated by chronically infected experimental animals. Limited air circulation in the animal holding facilities and the lower RH during the winter months further increased the possibility of virus transmission by the airborne route.

L. Foot-and-Mouth Disease

There is considerable evidence[120,199] from field studies for airborne spread of foot-and-mouth disease virus (FMDV). It has also been shown that FMDV can survive well in air when the RH is high and the atmospheric temperature low.[94,96,97,200] Many FMDV outbreaks have been documented where the virus was transported over relatively long distances.[201] Predictive models have been used to successfully forecast and analyze the course of FMDV outbreaks.[202-204] FMDV-infected human subjects have been found to generate infectious aerosols of the virus during coughing, sneezing, talking, and breathing.[205]

M. Newcastle Disease Virus

Much of the evidence for the airborne spread of Newcastle disease virus comes from the elegant studies of Hugh-Jones et al.,[206] and it has been shown that use of high efficiency filters in poultry houses with air under positive pressure can prevent the acquisition of the disease by susceptible animals housed therein.[207]

N. Aujeszky's Disease

Gloster et al.[208] investigated a series of outbreaks of Aujeszky's disease which occurred in the United Kingdom from 1981 to 1982. Their findings suggested that 7 out of 11 outbreaks investigated could have resulted from airborne spread of the virus. Previous experimental[209] and field studies[208] lend some support to this hypothesis.

O. Marek's Disease Virus

This virus has been shown to be experimentally transmitted by the airborne route.[210-212] Further studies[164,213,214] have shown that virus replication occurs in the epithelia of feather follicles and large numbers of the virus are present on the feathers of infected birds and persist there in an infectious state for considerable periods. Under natural conditions, this will inevitably lead to the resuspension in air of infectious virus

with the strong possibility of airborne spread. Furthermore, dust from poultry houses has been shown to remain infectious for up to 4 weeks.[215] Forced ventilation of poultry houses could contribute to the airborne virus load from contaminated houses and promote the aerial spread of the virus, but use of certain air filters has been shown to prevent the natural spread of the infection.[164,216]

P. Enteric Viruses

The seasonality and patterns of spread of a number of enteric virus infections suggest that air may play a role in their transmission. As can be seen from Table 2, aerosol challenge of susceptible hosts with many types of entero- and adenoviruses can result in the development of respiratory infection. This mode of spread is particularly interesting in situations where the inhaled enteric virus subsequently causes acute gastroenteritis.[23] Infectious particles of certain types of entero- and adenoviruses have also been recovered from naturally occurring aerosols (Table 3). Very little epidemiological work has, however, been done thus far to determine the relative importance of air as a vehicle in the spread of enteric virus infections.

Q. Mumps and Rubella Viruses

In spite of the fact that both mumps[217] and rubella[218] are conventionally thought to spread by the airborne route, very little is known about the airborne survival and transmission of these viruses.

X. CONCLUDING REMARKS

In the industrialized world, the widespread use of potable water disinfection and general improvements in methods of food sanitation have drastically reduced the number of cases of those virus diseases known to be carried predominantly by these two vehicles. Similarly, the use of insecticides has resulted in the effective control of many arthropod-borne viral infections. On the other hand, airborne viral diseases may be generally more difficult to control[219] because (1) large numbers of infectious virus particles discharged by infected hosts may become airborne; (2) under certain environmental conditions, these viruses may remain infectious in the air for periods long enough for contact with susceptible hosts; and (3) compared to the oral route, smaller numbers of infectious virus particles are often required to initiate infection via the respiratory tract.[166]

Furthermore, increased population levels and a number of other facets of modern life may be increasing the levels of airborne viral pathogens in many parts of the world. Use of air recirculation to conserve energy resources and the increased crowding of humans inside institutional buildings with climate control increase the exposure of susceptible individuals to airborne viruses. Modern practices of animal husbandry also house large numbers of animals in relatively confined spaces and thus enhance exposure to infectious virus aerosols. Indoor air may therefore be expected to be relatively more important as a virus vehicle in the industrialized world than it is in developing countries. Spraying of human and animal wastes — which is known to generate infectious aerosols — to irrigate and fertilize crops is being practiced by an increasing number of communities and, in the developing world in particular, more and more hospital, veterinary, and research laboratories are routinely handling material containing potentially dangerous viruses.

In most natural settings, infectious virus discharged into the air is subject to immediate dilution. It can be surmized, therefore, that inhalation of such air by susceptible hosts may frequently result in exposure to small numbers of infectious virus particles,

thus leading to amplification of the virus in the infected host. Virus from subclinical as well as overt cases of airborne infection may well be carried to secondary hosts through direct contact or other vehicles, and thereby obscure the importance of air in disease transmission.

As is apparent from the information presented in this review, air can be an important vehicle in the spread of many viral infections although, in most individual outbreaks of disease, definitive evidence of airborne virus spread is difficult or impossible to obtain. However, our present knowledge and understanding of how viruses survive in air and by what mechanisms they are inactivated are very limited. Further insight into the mechanisms of airborne virus inactivation may be helpful in prevention and control of viral diseases. More systematic studies of airborne virus survival and inactivation would also be of use in establishing better methods for disinfecting viral aerosols which may be of particular use where air recycling is inevitable. On the other hand, knowledge of the factors which favor airborne virus survival could be used to advantage in the aerosol administration of live attenuated vaccines. Aerosol administration of such vaccines is already in use[212,220] and is likely to become increasingly important in the future. The recent interest in the use of genetically engineered viruses in environmental control raises many potential hazards. The capacity for airborne spread is in fact considered as a highly "desirable" attribute for a virus genetically altered by man.[221]

Many technical limitations exist in the quantitative recovery of infectious viruses from large volumes of air, and this has restricted attempts to assess the true role of air in the spread of viral infections. There is, therefore, an obvious need for an efficient, inexpensive, and quietly running large-volume sampler for routine monitoring of airborne microorganisms, particularly in institutional settings such as hospitals.

Virtually nothing is known about the response of the host to simultaneous exposure to infectious viruses and other airborne contaminants. Increased use of municipal wastes for spray irrigation is one example where exposure to bacteria, viruses, endotoxins, and chemicals may occur at the same time. There is already ample epidemiological and experimental evidence to suggest that respiratory viral infections may predispose the infected host to secondary invasion by other microorganisms.[222] Many viruses which have the potential for spread through air have been isolated from cases of acute bronchial asthma in children and are considered to play a key role in the etiology of this clinical condition.[223] Several industrial airborne particulates, among them lignite flyash,[224] have been shown to potentiate the action of certain types of viruses in vitro. In vivo studies are very limited but there are indications that certain airborne industrial pollutants may influence both the susceptibility to virus disease and the course of the infection.[225]

Air is a vital commodity and deterioration in its quality can lead to an involuntary exposure of humans and animals to harmful chemicals and microorganisms. Examples of the airborne spread of poisonous industrial chemicals and wastes to human populations in both the developed as well as the developing parts of the world are already too numerous to count. Whereas human and animal pathogenic viruses may inherently lack the potential to spread by outdoor air to the same extent, their capacity for transmission within the indoor environment cannot be overestimated. Therefore, the continued pursuit of this field of research is well warranted.

ACKNOWLEDGMENT

The authors gratefully acknowledge the help of Mrs. V. S. Springthorpe and Dr. C. M. Johnson-Lussenburg in the preparation of this manuscript.

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