CANADIAN THESES ON MICROFICHE

THÈSES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Canada

NL 339 (r. 86/09)

STUDIES ON THE AIRBORNE SURVIVAL OF ROTAVIRUSES AND A HUMAN CORONAVIRUS

A Thesis submitted to the

School of Graduate Studies

University of Ottawa

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Department of Microbiology and Immunology

School of Medicine

by

Mohammad Khalid Ijaz, D.V.M.

May,1985

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

IN THE NAME OF ALLAH, THE COMPASSIONATE, THE MERCIFUL

— O my Lord ! increase me in knowledge

(Surah Ta Ha, Section 16, 114. Al Quran)

Dedicated to my parents.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to Dr. Syed A. Sattar, supervisor of my thesis research, for his patience and encouragement throughout this study. I would also like to thank Dr. C. M. Johnson-Lussenburg, particularly for her guidance in the studies on the corona-virus. I am very grateful to Drs. E. Perry and K. Dimock for their generosity and many fruitful discussions.

As members of my thesis advisory committee, Drs. J. C. N. Westwood, D. A. Kennedy, and R. Nair always provided me with useful suggestions for experiments and constructive criticism of my work. Dr. Nair was also very helpful in the statistical analyses of the data for this thesis. I thank Dr. A. J. Springthorpe, Bell Northern Research Laboratories, Ottawa, for designing the computer program used in the analysis and plotting of the data.

Special thanks are due to all the members of Dr. Sattar's and Dr. Johnson-Lussenburg's laboratories for their assistance and cooperation during various phases of my experimental work. I am particularly grateful to Mrs. V. Susan Springthorpe and Mr. A. H. Brunner for their help.

I would like to thank all other members of the teaching and support staff and my fellow graduate students for providing me with a friendly and stimulating atmosphere to work in. I wish to express special appreciation to my colleagues Vito Scalia and Yasmin G. Karim for their friendship and moral support throughout my stay in Ottawa.

The financial assistance received from the World Health Organization, Health and Welfare Canada, School of Graduate Studies of this university, and Australian National Animal Health Laboratory is gratefully acknowledged.

SUMMARY

The effect of relative humidity (RH) and air temperature on the airborne survival of a human rotavirus (HRV) and a human coronavirus (HCV) was studied. Poliovirus (PV) type 1 (Sabin) was used as a reference. Tryptose phosphate broth (TPB), containing an antifoam agent and a fluorescent dye as a physical tracer, was used to suspend the virus to be aerosolized using a Collison nebulizer. The aerosols were held in a 300-L rotating drum and an all-glass impinger, containing TPB with an antifoam agent, was used for aerosol collection.

At 20±1°C, the half-life of HRV was 18.7±3.6 h, 27.8±10 h and 9.0±1.9 h at the low (30±5%), medium (50±5%) and high (80±5%) RH levels, respectively. At the same temperature, the half-life of HCV was 26.8±6.2 h, 67.3±8.2 and 3.3±0.16 at the low, mid and high RH levels, respectively. At 6±1°C, their survival was further enhanced at the mid and low RH, but there was no increase in the half-life of HRV at 80±5% RH; at this temperature there was, however, a dramatic improvement in the half-life (86.0±5.3 h) of HCV at the high RH. When PV was mixed with either HRV or HCV and the aerosolized mixture held at 20±1°C, no infectious PV could be detected at the low and mid RH, but at the high RH the half-life of PV was 9.0±1.9 h. The airborne survival of rotaviruses of simian, bovine and murine origin was also studied and their behavior in this regard was found to be similar to that of HRV.

Although rota- and coronaviruses are important pathogens of humans and animals, very little is known about the vehicles of their spread in nature. The findings of this study suggest that, at least under certain environmental conditions, air may act as a vehicle in their direct or indirect spread. These data may also help in understanding the seasonal nature of the outbreaks caused by these viruses.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AGI	All-glass impinger
ASFV	African swine fever virus
BAdV-1	Bovine adenovirus type-1
BPI-3	Bovine parainfluenza virus type-3
BUdR	5-Bromodeoxyuridine
°C	Degrees centigrade
cm	Centimeter
CsCl	Cesium chloride
DEAE-Dextran	Diethylaminoethyl-dextran
EAV	Equine arthritis virus
EBSS	Earle's balanced salt solution
EDIM	Epizootic diarrhea of infant mice
EDTA	Ethylenediaminetetraacetic acid
EHV-1	Equine herpesvirus type-1
EMC	Encephalomyocarditis
ERV-1	Equine rhinovirus type-1
FCV	Feline calicivirus
FCS	Fetal calf serum
FHV	Feline herpesvirus
FMD	Foot-and-Mouth Disease
HCV/229E	Human coronavirus 229E
IBR	Infectious bovine rhinotracheitis
IBV	Avian infectious bronchitis virus
L	Litre
LVAS	Large volume air sampler

m	Meter
MEM	Minimal essential medium (Eagle's)
mg	Milligram
mL	Millilitre
NDV	Newcastle disease virus
nm	Nanometre
OAF	Open air factor
PBS	Phosphate buffered saline
PFU	Plaque forming units
PI-3	Parainfluenza-3
ppm	Parts per million
RH	Relative humidity
rpm	Revolutions per minute
S.D.	Standard deviation
SV_{40}	Simian $virus_{40}$
TPB	Tryptose phosphate broth
uCi	Microcurie
ug	Microgram
um	Micrometer
UV	Ultraviolet
VEE	Venezuelan equine encephalitis
VEV	Vesicular exanthema virus

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

AEROSOLS AND THE SPREAD OF INFECTIOUS DISEASES

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 1930's. 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 5 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. In view of this, a great deal of work has also been conducted to determine which factors promote or retard the survival of infectious agents in air, and what preventive and control measures are likely to be effective in safeguarding against airborne infections.

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 particle of 100 um diameter 10 seconds to fall through the height of an ordinary room (about 3 meters); particles with diameters of 40, 20 and 10 um would require 1, 4 and 17 minutes, respectively, to settle out under the same set of conditions. On the other hand, particles 1-3 um in diameter will remain suspended almost indefinitely, especially if they are periodically elevated by air currents (Guyton, 1947; Knight, 1973).

Many common and natural activities regularly result in the generation of aerosols. For example, sneezing, coughing and even speaking by persons carrying viruses in their mouth and respiratory tract frequently lead to the aerosolization of viruses (Winkler, 1973).

The particles produced during sneezing and speaking (particularly when pronouncing sibilants) are generally larger and most of them rapidly settle out of air. In contrast to this, coughing is known to produce more small-particle aerosols which are potentially better suited for the airborne spread of viral infections.

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 are referred to as "droplet nuclei" (Wells and Stone, 1934), and if the biological agents in them are not damaged by the drying process, then they are 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 (Hatch, 1961; Stuart, 1973; Brain and Valberg, 1979; Teshima et al., 1981). The actual site of their retention in the respiratory tract is determined principally by the size of the particles being inhaled.

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 Fig.1.

PARTICLE SIZE AND VIRUS RETENTION IN THE RESPIRATORY TRACT

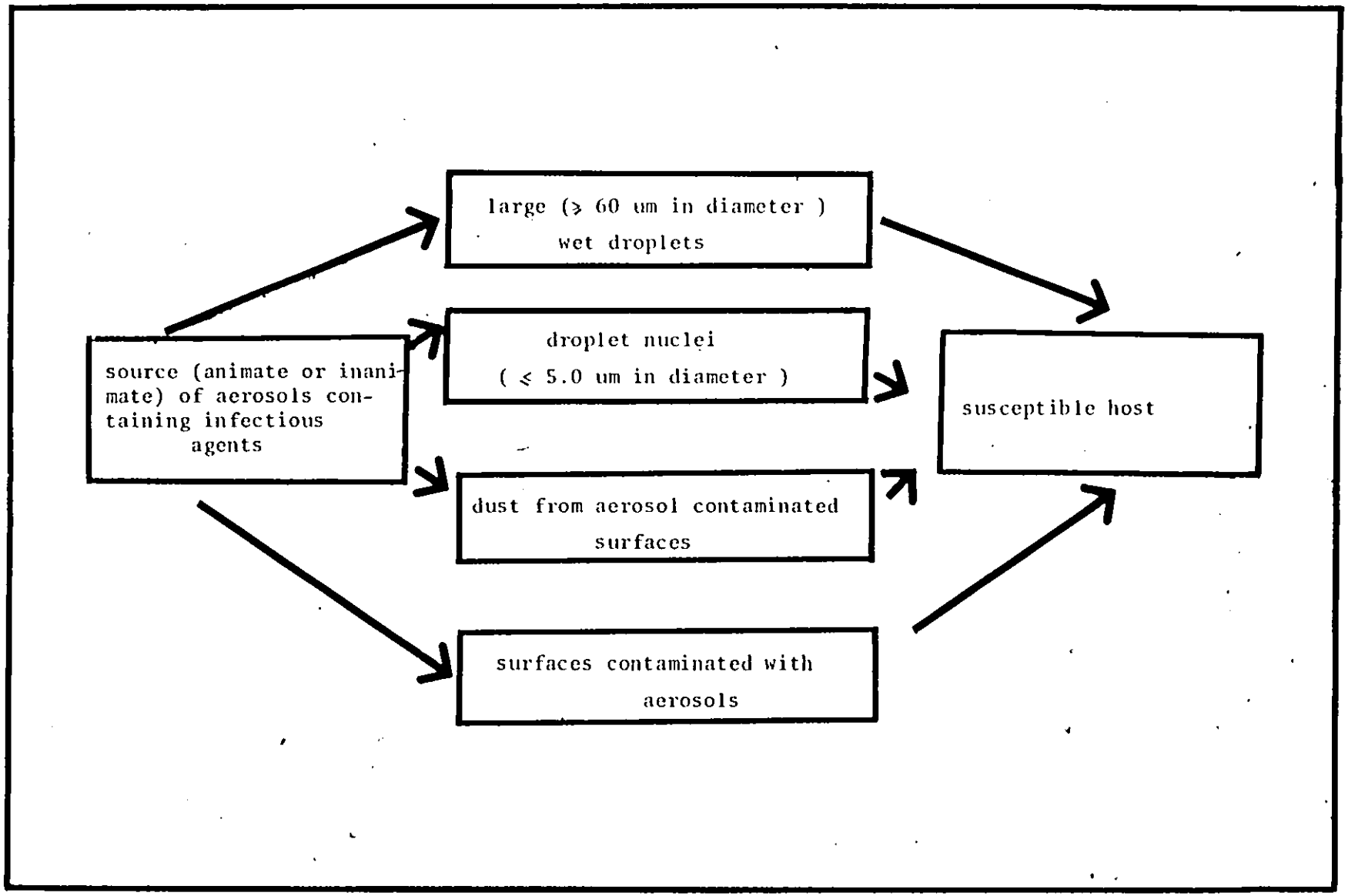
The particle size of virus-containing aerosols is important in determining their physical stability as well as the site and extent of their retention in the respiratory tract. Therefore, some knowledge of the particle diameters of viral aerosols is considered essential in understanding airborne transmission of viral infections.

A human adult breathes between 10 and 20 thousand litres of air per day (Bell et al., 1963). Such air may contain particles of many types and sizes. However, from the point of view of infectious disease spread by the airborne route, particles of particular importance fall in the size range of 0.1-60.0 um in diameter (Stuart, 1973).

The greatest degree of deposition in the alveoli of human lungs occurs when the inhaled particles are in the 1-3 um range; it decreases to a minimum for particles of 0.25 um. For particles below 0.25 um, alveolar deposition again increases due to Brownian movement (Brown et al., 1950; Hatch 1961; Lippman and Albert, 1969; Stuart, 1973; Morrow, 1980). Fuchs (1964) observed that 82, 28 and 51% of the particles 1.0 um, 0.1-0.3 um and 0.03 um, respectively, were retained in alveoli or alveolar passages.

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 (Slote, 1976). This could conceivably result in the spread of certain infections of the gastrointestinal tract by the airborne route (Flewett, 1982).

Figure 1. Possible ways of spread of infections through the airborne route.


large (≥ 60 um in diameter)
wet droplets
droplet nuclei
(≤ 5.0 um in diameter)
source (animate or inanimate) of aerosols containing infectious agents
susceptible host
dust from aerosol contaminated surfaces
surfaces contaminated with aerosols

GENERATION, STORAGE AND COLLECTION OF VIRAL AEROSOLS

Experimental generation of viral aerosols can be carried out by the DeVilbis #40 or the Vaponefrin nebulizers (Larson et al., 1976), but the most popular device for this purpose is the Collison nebulizer (May, 1973). More than 90% of the airborne particles generated by it are <5 um in diameter and they are, therefore, potentially suited for retention in the respiratory tract. This nebulizer has been used to generate aerosols of many types of lipid-containing (Guerin and Mitchell, 1964; Mitchell et al., 1968; Akers et al., 1973; Hugh-Jones et al., 1973; Fairchild, 1974; Schaffer et al., 1976; Scott and Sydiskis, 1976; Walker et al., 1976) as well as non-lipid-containing viruses (Guerin and Mitchell, 1964; Couch et al., 1965; 1966a; 1966b; Gerone et al., 1966; Benbough, 1971; Strange et al., 1972; Barlow and Donaldson, 1973; Donaldson, 1973; Donaldson and Ferris, 1975; Adams et al., 1982; Moe and Harper, 1983).

Compressed air is used for the generation of aerosols by the devices mentioned above. As the air expands through the restricting orifices, the suspension to be aerosolized is broken into particles of many sizes. A major proportion of these are too large to be carried the outgoing air stream, and they consequently drop back into the suspension. Particles of smaller size can remain airborne for longer periods and may be carried out of the nebulizer in the air jet.

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). The AGI (Wolf et al., 1959) has been used in studying the aerosol stability of the following types of vertebrate viruses: Colorado tick fever, vesicular stomatitis (VSV), encephalomyocarditis (EMC), neurovaccinia (Watkins et al., 1963); Rous sarcoma (Webb et al., 1963); adenovirus types 4 and 7, human parainfluenza (Miller and Artenstein, 1967); Newcastle disease (NDV) (Songer, 1967); infectious bovine rhinotracheitis (IBR) (Songer, 1967; Elazhary and Derbyshire, 1977); bovine parainfluenza type 3, bovine adenovirus type 3 (Elazhary and Derbyshire, 1979a; 1979b); rinderpest (Hyslop, 1979); poliovirus type 1 (Sabin), rhinovirus type 14, human coronavirus 229E (HCV/229E) (Park, 1980). Here it should be noted that the AGI is considered relatively inefficient for the collection of viral aerosols where a large proportion of the airborne particles are smaller than 0.1 um in diameter (May and Harper, 1957). Such small particles have a tendency to escape entrapment in the aerosol collection fluid.

May and Druett (1953) have described a simple device called a pre-impinger, which, when attached to the front of an impinger, divides the total aerosol sample into two particle size fractions by means of size selective impingment. The cut off between the two fractions is set at 4 um to simulate nasal penetration; it is believed that the particle retention by the pre-impinger is similar to that of the nasal passage, while the material in the impinger is similar to that reaching the lungs.

Impaction devices for the collection of aerosols are named after the type of opening through which the aerosol is collected. These samplers include: (a) the slit sampler (Wolf et al., 1964) 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, (b) the sieve sampler (Wolf et al., 1964) where the airborne particles accelerate through small holes and impact on the collection surface, (c) the stacked-sieve or Anderson sampler (Anderson, 1958) which consists of 6 sieves stacked one on top of the other; 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 (Guerin and Mitchell, 1964; Couch et al., 1965; Peto and Powell, 1970; Lembke et al., 1981; Bausum et al., 1982).

Certain limitations inherent in the above-mentioned impaction devices make them unsuitable for use in the routine study of airborne viruses. However, such devices are extremely valuable in the study of other types of airborne microorganisms as well as in the determination of the basic characteristics of aerosols in general.

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 aerosols to susceptible experimental hosts. The apparatus most commonly used for aerosol challenge studies in animals is the one designed by Henderson (1952). It can act as a continuous source of the infectious agent at a constant cloud density under controlled conditions of RH and temperature. By placing appro-

priate test animals in the aerosol cloud, they can then be exposed to the infectious agents for susceptibility and transmission studies. More recently, McVicar and Eisner (1983) reported a relatively simple and inexpensive means of exposing cattle of all ages to virus aerosols. Survival characteristics of an airborne infectious agent can be studied in another type of aerosol maintenance chamber known as the dynamic aerosol toroid or rotating drum (Goldberg et al., 1958). 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 "fallout" on the walls of the drum. Goldberg (1971) has presented in detail those factors that govern the behavior of aerosolized particles in the rotating drum.

May and Druett (1968) and Druett (1971) have described a microthread technique for studying the viability of microorganisms 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 large volume air sampling (LVAS) devices are a relatively recent development in the study of airborne infectious agents. Spendlove and Fannin (1982) have recently reviewed the literature on these devices.

FACTORS GOVERNING VIRUS SURVIVAL IN THE AIRBORNE STATE

Factors that influence the survival of airborne viruses include: (1) relative humidity and atmospheric temperature (2) atmospheric gases and aerosolized chemicals, (3) nature and composition of the spray and collection fluids , (4) irradiation , and (5) the genetic make-up of the virus.The following is a summary of the published information on how the above-mentioned factors affect the airborne survival of a variety of vertebrate viruses.

1) Relative Humidity and Air Temperature:

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 the epidemiology of many viral diseases. A summary of the available information on how atmospheric temperature and RH affect the survival of various airborne viruses has been presented in Table 1.

It is generally believed that there is a simple correlation between the chemical composition of a given virus and the influence of RH on its airborne survival (Hemmes et al., 1960). In other words, lipid-containing viruses are thought to survive better at low levels of RH and that the high RH levels are considered to be more conducive to the airborne survival of viruses which are lipid-free (de Jong et al., 1973; Hyslop, 1973; Donaldson and Ferris, 1976;). However, it is now known that there are important exceptions to this rule.

Under certain experimental conditions, some types of viruses have been found to survive well at high and low RH levels, but were sensi-

TABLE 1

EFFECT OF TEMPERATURE & RELATIVE HUMIDITY ON THE SURVIVAL OF AEROSOLIZED VIRUSES

REFERENCE	VIRUS(ES)	Temperature °C	Relative Humidity %	PERIOD OF AEROSOL AGING	REMARKS
Loosli et al. (1943)	influenza A	27-29	23 48 89	24 hr.	Experiments were performed in a room of 800 cu. ft. 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.
Schechmeister (1950)	influenza A (W.S. strain)	?	30-70	?	Highest levels of airborne infectious virus were recovered at the two RH extremes (32 and 68%).
Hemmes et al. (1960)	influenza (PR8), poliovirus type 1 (CSL)	?	0-100	2 min.	For influenza virus, inactivation rate high at 50-90% RH and low at 15-40% RH. For poliovirus 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.
Harper (1961)	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 hrs.	For all the virus types studied, survival at all RH ranges tested was better at the lower than at the higher temperature. Poliovirus survived best at high RH levels, whereas the other 3 viruses tested survived best at low RH levels.
Miller et al. (1963)	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.
Webb et al. (1963)	pigeonpox and Rous sarcoma	?	0-100	5 hrs.	Pigeonpox virus was found to be stable in aerosols and was affected little by RH. Rous sarcoma virus was extremely sensitive to RH and survived best at RH levels above 70%. Inositol at a final concentration of 60% in the spray fluid was able to prevent inactivation of Rous sarcoma virus at RH levels below 70%.
de Jong and Winkler (1964)	measles (Edmonston strain)	20-21	10-100	?	The virus was sprayed in the 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.

REFERENCE	VIRUS(ES)	Temperature °C	Relative Humidity %	PERIOD OF AEROSOL AGING	REMARKS
Akers et al. (1966b)	encephalomyo-carditis (Mengo, Maus Elberfeld and Columbia SK)	16 26	5-95	6 hrs.	At 16°C, virus inactivation during the first 5 minutes 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.
Songer (1967)	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.
Miller and Artenstein, (1967)	adenovirus types 4 and 7 and para-influenza type 3	23.7	20 50 80	6 hrs.	Both types of adenovirus were most stable at 80% RH whereas parainfluenza was most stable at 20% RH.
de Jong and Winkler (1968)	poliovirus 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 poliovirus.
Mitchell et al. (1968)	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.
Benbough (1969)	Semliki Forest	22	20-90	24 hrs.	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.
de Jong (1970)	polio and encephalomyo-carditis (EMC)	20	70-90	?	Inferred that virus inactivation at 40% RH is due to deterioration of the protein coat.
Mayhew and Hahon (1970)	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.

REFERENCE	VIRUS(ES)	Temperature °C	Relative Humidity %	PERIOD OF AEROSOL AGING	REMARKS
Davis et al. (1971)	adenovirus type 12	28-29.5	32, 51 and 89	20 min.	The virus was found to survive best at the highest level of RH tested (89%).
Ehrlich and Miller (1971)	VEE	-40 to +49	18-90	1-2 hrs.	The biological decay rate of airborne VEE was not markedly affected in the temperature range -40 to 24°C at any RH tested (18-90%). However, at 49°C a significant increase in decay rate was observed.
Barlow (1972a)	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.
Donaldson (1972)	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.
Mitchell and Querin (1972)	influenza A, (human, avian, swine and equine strains)	21	15	39 hrs.	Avian and equine strains were considerably more resistant to decay than those derived from human or swine sources.
Akers et al. (1973)	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%).
Donaldson (1973)	foot-and-mouth disease (2 strains)	18-23	10-100	1 hr.	Viruses aeroslized 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.
de Jong et al. (1975)	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.
Donaldson and Ferris (1976)	feline herpesvirus (FHV), feline calicivirus (FCV), infectious bovine rhinotracheitis (IBR) virus	18-23	20-80	1 hr.	ASFV and PI-3 viruses survived well at all RH levels tested when tested only 1 second after aerosolization. However, after 5 mins. of storage, both these viruses were found to be sensitive to high RH. The other lipid-containing viruses (EAV, VSV, FHV, EMV-1 and IBR)

REFERENCE	VIRUS(ES)	Temperature °C	Relative Humidity %	PERIOD OF AEROSOL AGING	REMARKS
	parainfluenza-3 (PI-3), equine arteritis virus (EAV), equine herpesvirus 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)				were also unstable when stored as aerosols in high RH. ERV-1, a picornavirus, was the only virus which survived well at high RH but poorly on exposure to dry conditions. The caliciviruses (VEV and 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 non-lipid 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.
Schaffer et al. (1976)	influenza (WSNH strain)	21	20-80	1 hr.	The aerosolized virus was maximally stable at low RH (<40%), minimally stable at mid-range RH (50-70%), and moderately stable at high RH (>80%). 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.
Elazhary and Derbyshire (1977)	infectious bovine rhinotracheitis (IBR)	6 or 32	30 or 90	3 hrs.	The virus was found to be more stable when aerosolized from nasal secretions and at high RH (>90%). Low temperature and high RH were the most favourable for short-term survival.
Elazhary and Derbyshire (1979a)	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.
Elazhary and Derbyshire (1979b)	bovine adenovirus type-3 (BAdV-3)	6 or 32	30 or 90	3 hrs.	Virus inactivation was usually more rapid at 30% RH than at 90% RH, and at 32°C than at 6°C. Glucose was found to protect the virus during spraying and

REFERENCE	VIRUS(ES)	Temperature °C	Relative Humidity %	PERIOD OF AEROSOL AGING	REMARKS
					amino acids in EMEM were thought to be more protective during aerosol aging.
Hyslop (1979)	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%).
Adams et al. (1982)	reovirus type-1 (Lang strain)	21-24	25-95	3 hrs.	Aerosol stability of infectious and potentially infectious virus particles was found to be maximal in the 85-95% RH range. An increase in recovery of the aerosolised virus was observed upon prehumidification.
Moe & Harper (1983)	calf rotavirus (U.K. strain)	10 20 30	20 50 90	2 hrs.	Virus stable at low and high RH. Low temperature more conducive to virus survival in air.
Stephenson et al. (1984)	Lassa virus (Josiah strain)	24 32 38	30 55 80	1 hr.	The virus was to be stable in aerosol form, particularly at low RH (30%) level. Biological half-life at both 24 and 32°C (30% RH) was found to be 54.6 and 29.1 minutes respectively. This was considered sufficient for aerosol dispersion of the virus to considerable distances in natural situations.

tive to inactivation at the mid RH range. Examples of such viruses are VSV (Watkins et al., 1963), Langat virus (Benbough, 1971), mengovirus (Akers and Hatch, 1968), two types of caliciviruses (Donaldson and Ferris, 1976), and influenza virus (Schaffer et al., 1976).

Humidification of aerosols immediately prior to their collection has been shown to increase the recovery of infective particles of certain types of viruses (Hatch and Warren, 1969; Warren et al., 1969; Dubovi and Akers, 1970; Benbough, 1971; Barlow, 1972b; Trouwborst and Kuyper, 1974; de Jong et al., 1975; Adams et al., 1982;). This indicates that rehydration of airborne virus particles leads to their reactivation. Infective yields of certain other vertebrate virus aerosols, such as vesicular exanthema virus (Ferris and Donaldson, 1980), remained unaffected by pre-humidification. In contrast to this, pre-humidification has also been shown to decrease the efficiency of recovery of some types of viruses (Warren et al., 1969).

As can be seen from Table 1, most of the aerobiological studies on viruses have been carried out at room temperature (20-24°C). However, data generated in studies where the air temperature was maintained either above or below this range, and the RH level was also varied, show that the combined effect of these two factors on virus infectivity is complex and highly variable from virus to virus.

Harper (1961,1963) observed that the survival of airborne vaccinia, influenza and VEE during six hours in aerosol was greatest at low temperature (10°C) and the influence of RH on virus infectivity at this temperature was negligible; at the highest temperature studied (32°C), the viruses survived least well and this observation led the author to suggest that "the higher the temperature, the greater the influence of

RH". As far as initial viability is concerned, Harper (1961) reported that influenza and vaccinia viruses were not influenced by temperature and RH, while the viability of VEE had a tendency to be dependant on both.

Songer (1967) studied the airborne stability of NDV aerosols held at 4, 23, and 37°C. At 23°C virus survival was better at 10% RH than at 35% or 90% RH. At 10% RH, airborne NDV survived equally well at the three temperatures tested, whereas at higher RH levels, the lower temperature favored the survival. SV_{40} was found to be stable in the RH range 22-80% at 21°C, but aerosols of this virus maintained at 32°C were inactivated within 60 min at mid-range RH levels (Akers et al.,1973). The unusual airborne stability of this virus at 21°C over a broad range of RH indicates that potentially biohazardous situations could occur under laboratory conditions if this virus is accidentally aerosolized.

Elazhary and Derbyshire (1977; 1979a; 1979b) studied the aerosol stability of IBR virus, bovine parainfluenza virus type 3 and bovine adenovirus type 3. They found that the most favourable conditions for short term survival of these viruses in the aerosol form seemed to be low temperature and high RH. It was suggested earlier (Derbyshire and Roberts, 1968) that this may be the reason for the higher number of field infections of cattle with parainfluenza virus type 3 during autumn and early winter in England.

It is not yet clearly understood by what mechanism(s) air temperature and RH promote or retard the survival of airborne viruses. Akers (1969) 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 (1969) also suggests that the effect of air temperature is secondary in the inactivation of airborne viruses.

2) Atmospheric Gases and Aerosolized Chemicals:

The presence of certain chemicals in the gaseous state has been shown to inactivate aerosolized viruses. 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. For example, in one of the earliest studies in this field propylene glycol vapors were found to be effective in preventing infection of mice exposed to experimentally generated influenza virus aerosols (Henle et al., 1942). It is also conceivable, however, that under certain conditions, some naturally occurring or artificially generated gaseous substances could protect virus infectivity in air.

Berendt et al. (1971) have shown that a low concentration of sulphur dioxide in air (0.4 ppm) is sufficient to inactivate airborne VEE virus, but concomitant irradiation with simulated sunlight reduces the virucidal properties of the gas. Higher concentrations of sulphur dioxide (3.6 ppm) were able to inactivate the airborne virus even when it was irradiated with artificial sunlight (Berendt et al., 1972), and this occurred more rapidly at 60% RH than at 30% RH. The rate of virus 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 sulphur dioxide inactivates airborne viruses is not known, it may be attributable to the formation of sulphuric acid in moist air. In artificial sunlight, sulphur dioxide

may be expected to be somewhat photolabile, and therefore, at low levels of sulphur dioxide, the virucidal species may be reduced beyond its effective level.

Ehrlich and Miller (1972), 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.

Slobodenyuk and Karpukhin (1970) investigated the effect of hydrogen peroxide, chloramine and hexylresorcine on airborne adenovirus type 3, poliovirus type 3 and coxsackievirus type B1. The minimum concentrations of these compounds needed to bring about a 99.9% reduction of all three of the viruses in 30 minutes were 10-20, 5-10 and 5 mg/m^3, respectively. However, in the same study, much higher concentrations of the same disinfectants were required to bring about a 99.9% reduction in virus titre on a contaminated surface.

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. It has also been suggested that the behavior of viruses in the indoor environment would be very different from that in open air, the difference being due to the open air factor (OAF) (Druett and May, 1968; May et al., 1969). The OAF is believed to be a complex formed by ozone and gaseous unsaturated hydrocarbons similar to those from internal combustion engines (Spendlove and Fannin, 1982). Hood (1971)

has designed an indoor system for studying the survival of airborne microorganisms in closed conditions to allow the study of the effect of outdoor air.

Benbough and Hood (1971) found that inactivation of Semliki Forest virus in the airborne state was considerably enhanced in outdoor compared to indoor air. On the other hand, when the effect of day light and the OAF on certain strains of FMD virus was studied (Donaldson and Ferris 1975), neither daylight nor the OAF could influence the airborne survival of this virus provided that the RH was higher than 60%.

4) Nature and Composition of Spray and Collection Fluids:

It is well established that the composition of the fluid from which the virus is aerosolized can greatly influence its subsequent airborne stability (Webb, 1959; 1963 Harper, 1961; 1965; Akers, 1969; 1973; Dubovi and Akers, 1970; Benbough, 1971; Donaldson, 1978; Elazhary and Derbyshire, 1977; 1979a; 1979b;). In view of the above, 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.

Whereas 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, nasal secretions, milk and feces. Donaldson (1972, 1973) reported that when FMD virus was aerosolized from bovine milk or fecal slurry, it was more stable than when bovine saliva was used as the spray medium. This difference was believed to be due to the comparatively high pH (8.9-9.1) of saliva (Barlow,

1972a). Barlow and Donaldson (1973) also compared the effects of artificial and natural spray fluids on the airborne stability of FMD virus. They found that at high RH, virus sprayed from bovine milk, feces, nasal fluid and cell culture supernatant fluid was more stable than that aerosolized from bovine saliva. The relative instability of the virus sprayed from saliva was due to an undialysable component which was stable at 60°C, but could be inactivated by heating at 70°C.

Elazhary and Derbyshire (1977) 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 with 90% RH. However, bovine adenovirus was found to survive better when aerosolized from MEM than when the aerosol was generated from nasal secretions of a seronegative calf (Elazhary and Derbyshire, 1979a). In this case, glucose and amino acids in the MEM were considered to protect the virus during spraying and aerosol aging, respectively. Peptone and apolar amino acids were also shown to be protective to airborne EMC and Semliki Forest viruses (Trouwborst et al., 1974). The surface protective role of peptone was further substantiated by the work of Donaldson and Ferris (1976) using IBR.

A slight change in the spray fluid can lead not only to a change in the rate of biological decay of viruses, but can also influence their response to inactivation by RH (Watkins et al., 1963; Akers et al., 1966a; de Jong 1967).

No additive has yet been found which is capable of completely

stabilizing the infectivity of airborne viruses. However, the presence of certain types of chemicals in the spray or collection medium has 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. (1963) found that the rapid inactivation of aerosolized Rous sarcoma virus at low RH could be prevented by the presence of inositol in the spray fluid. A similar stabilizing effect of this chemical was noted for aerosols of influenza, Langat, Semliki Forest and FMD viruses (Barlow, 1972b; Akers, 1973;). On the other hand, inositol was not found to influence the airborne survival of a number of other virus types, such as EMC, VEV- and polioviruses. (Akers, 1973; Benbough, 1971; Ferris and Donaldson, 1980).

Park (1980) compared 10% glycerol and 0.1% bovine serum albumin for their efficacy in stabilizing aerosolized poliovirus type 1 (Sabin). Glycerol was found to be superior in this regard and this protective effect of glycerol may be through the prevention of dehydration of the virus at the low and medium RH levels.

The presence of protein-rich substances such as serum in the spray fluid appears to slow down the evaporation of water from the aerosolized particles due to the hydration of the protein molecules. This is believed to be the basis for the enhanced survival of aerosolized FMD virus at a wide range of RH when it was sprayed from bovine milk (Donaldson, 1973). Other proteinaceous substances such as bovine serum albumin and peptone have also been found to protect the infectivity of airborne Langat (Benbough, 1969), Semliki Forest (Dubovi and Akers, 1970; Benbough, 1971) and vaccinia viruses (Harper, 1963a).

It seems that the presence of proteins in the spray fluid is not

necessarily protective for all types of airborne viruses. This is exemplified by the studies of Harper (1963) on poliovirus and of de Jong (1975) using EMC virus.

In addition to studying the influence of RH and composition of the spray fluid, Schaffer et al. (1976) tested the effect 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.

4) Irradiation:

Following the early studies of Wells and Brown (1936) and Wells and Henle (1941), Edward et al. (1943a) 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 wavelenth of 253.7 nm. Similar inactivation by UV light was observed for airborne Rous sarcoma virus (Webb, 1963), coxsackie B1, Sindbis, influenza A and vaccinia viruses (Jensen, 1964). Adenovirus type 2 was shown to be somewhat more resistant to UV inactivation under the same conditions (Jensen, 1964).

Berendt and Dorsey (1971) 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 sulphur dioxide at high RH levels (Berendt et al., 1971; 1972). Attempts have been made to use

such lethal effects of UV to interrupt virus transmission by the airborne route. UV disinfection of airborne influenza virus has been shown to be effective in preventing infection of mice exposed to experimentally-generated aerosols of the virus (Henle et al., 1942). Riley (1977) 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 been reported (Wells et al., 1942; Perkins et al., 1947) and it appeared to be effective.

5) Genetic Make-up of the Virus:

It has been demonstrated that different strains, types or subtypes of the same virus may behave differently in the airborne state. This is supported by the work of Hyslop (1970) on two different strains of type SAT_1 of FMD virus. He observed that, under the same set of experimental conditions, strain TUR 323/62 decayed more slowly in the airborne state than strain SA 13/61. This observation was further substantiated by the work of Donaldson (1972), who showed that O_1 PACHECO strain of FMD virus survived better than O_1 BFS 1860 at 70% and 55% RH levels (18-23°C). Similarly, Hood (1963) found that the maximal initial loss of two different strains of influenza virus type A occurred at two different RH levels; strain PR8 decayed more rapidly at the high RH, whereas, strain Singapore lost its infectivity faster at the medium RH level. When tested under the same set of experimental conditions, airborne adenovirus type 4 was found to decay faster than adenovirus type 7 (Miller and Artenstein, 1967).

In contrast to the above, the studies of Akers et al.(1966a) have shown that 3 different strains of cardioviruses (Mengo, Maus Elberfeld

and Col SK) behave very similarly when aerosolized and held under the same experimental conditions.

MECHANISM OF INACTIVATION OF VIRUSES IN THE AIRBORNE STATE

Our knowledge of the mechanism(s) leading to the inactivation of airborne vertebrate viruses is still very rudimentary. However, a number of hypotheses have been put forward in this regard (Webb, 1959, 1963; Webb et al., 1963; Bather and Webb, 1966; de Jong et al., 1973; Trouwborst et al., 1973).

Webb et al. (1963) suggested that the rapid inactivation of aerosolized Rous sarcoma virus at the low RH was due to the removal of the stabilizing water molecules from the nucleoprotein of the virus and that the presence of inositol in the spray fluid prevented this water loss, thereby leading to the enhanced survival of the airborne virus.

In view of the above hypothesis, attempts have been made to detect infectious nucleic acid in virions after their inactivation in the airborne state. It was demonstrated that following such inactivation infectious RNA could be extracted from Mengo virus (Akers and Hatch, 1968), EMC virus (de Jong, 1970; de Jong et al., 1974) and poliovirus (de Jong et al., 1973). Studies carried out with certain bacterial viruses yielded similar results (Dubovi, 1971; Trouwborst et al., 1974).

Although the denaturation of the protein components of aerosolized virus particles could also lead to their inactivation, very little experimental work has been done to elucidate this phenomenon. However,

studies with enzymes such as trypsin have shown that the denaturation of protein molecules in the airborne state is humidity dependent (Trouwborst et al., 1973).

Here it should be noted that the type of aerosolized virus which may undergo inactivation by more than one mechanism and the actual mechanism of its inactivation may depend on the composition of the spray fluid, RH and air temperature. The observations of Benbough (1969) with aerosolized Semliki Forest virus are a case in point; 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 compound added to the spray fluid protected the infectivity of the virus at high and low RH levels, respectively.

ROTAVIRUSES

Rotaviruses are spherical, double-stranded RNA viruses with a particle diameter of about 70 nm. They are among the most important causes of acute diarrhea in both human and animal neonates (Brandt et al., 1979; Holmes, 1979; Kapikian et al., 1979; Barnett, 1983; Estes et al., 1983; Cukor and Blacklow, 1984). During epidemics of acute diarrhea in infants and children, both in tropical and temperate areas of the world, up to 75% of the cases have been found to be due to rotaviruses (Holmes, 1979). It has been estimated that members of this viral group are responsible for more than five million infant deaths every year in the Third World (Flores et al., 1984). Rotavirus diarrhea is also commonly associated with mortality in newborn animals (McNulty, 1978).

There is mounting evidence to show that rotaviruses can cause acute diarrhea in human adults as well (Von Bonsdorff et al., 1976; Zissis et al., 1976; Lycke et al., 1978; Morens et al., 1979; Echeverria et al., 1983). Persons over 60 year of age appear to be particularly susceptible to infection by these agents (Elias, 1977; Cubitt and Holzel, 1980).

Outbreaks of rotaviral diarrhea frequently occur in pediatric (Brandt et al., 1979; Kapikian et al., 1981; Bryden et al., 1982) as well as other (Totterdell et al., 1976; Murphy et al., 1977; Holzel et al., 1980) hospitals. A prospective study has shown rotaviruses to be among the important etiological agents of infectious gastroenteritis in patients with bone marrow transplants (Yolken et al., 1982); the mortality rate among such infected patients was 55% as compared to 13% in

those who had remained uninfected.

Apart from increased mortality, nosocomial outbreaks of rotaviral diarrhea have been shown to cause significant morbidity and add greatly to the total cost of hospitalization for each affected individual. In a U.S. study, the increased hospitalization, diagnostic and therapeutic measures were found to result in an additional mean cost of U.S. $835.00 per infection (Ryder et al., 1977).

Outbreaks of rotaviral gastroenteritis are by no means restricted to hospitals. Such outbreaks are also known to occur in nursing homes (Fauvel et al., 1980; Halvorsrud and Orstavik, 1980; Marrie et al., 1982), day-care centres (Haug et al., 1978; Pickering et al., 1981), schools (Hara et al., 1976; Sutmoller et al., 1982) and in the community-at-large (Freij et al., 1978; Lycke et al., 1978; Morens et al., 1979; Echeverria et al., 1983). In such outbreaks, secondary person-to-person spread of the infection is known to occur (Kim et al., 1977; Morens et al., 1979). This is particularly important when children who contract rotaviral diarrhea in day-care centres or schools can act as sources of infection for their families (Haug et al., 1978; Pickering et al., 1981; Grimwood et al., 1983).

Rotaviruses also represent a potential health threat in the work environment. Published data are available (Hildreth et al., 1981) to show that, at least in the hospital setting, medical and nursing staff frequently contract the infection during nosocomial outbreaks of rotaviral gastroenteritis. With the advent of techniques for the in vitro cultivation of field strains of human rotaviruses (Urasawa et al., 1981) increasing numbers of hospital and research laboratories are becoming involved in their isolation and study. Rotaviruses are known

to be present in domestic sewage in very large numbers (Smith and Gerba, 1982; Raphael et al., 1985). This, therefore, represents a potential hazard to the health of those working in waste treatment establishments. There are, however, no published studies of work-related rotaviral infections in laboratory personnel or those working in waste treatment industry.

Modes and Vehicles of Spread of Rotaviruses:

Rotaviruses are excreted in very large numbers (>10^{10} particles per gram) in the feces of infected individuals. Such fecal matter represents the principal source of environmental contamination with rotaviruses. Recent studies using human volunteers (Kapikian et al., 1983) have shown that the ingestion of rotavirus contaminated material can lead to infection. No systematic studies have, however, been done to determine the relative importance of various common vehicles in the spread of rotaviral infections. The evidence presently available in this regard is, therefore, mostly indirect and circumstantial.

In certain outbreaks of rotaviral diarrhea in the general community, food (Hara et al., 1976) and water (Lycke et al., 1978; Sutmoller et al., 1982; Harris et al., 1983) appeared to be the vehicles for virus spread. Recently published studies have also demonstrated that rotaviruses could retain their infectivity for several weeks in raw river water (Raphael et al., 1985) and conventionally-treated drinking water (Sattar et al., 1984).

The capacity of rotaviruses to spread is further enhanced by their resistance to inactivation by a number of commonly used chemical disinfectants and antiseptics (Snodgrass and Herring, 1977; Tan and Schnagl,

1981; Sattar et al., 1983; Raphael et al., 1984). Many of the disinfectants tested in the above-mentioned studies are routinely used in hospitals, schools, microbiological laboratories, food processing and agricultural establishments and the home environment.

The studies of Woode and Bridger (1975) have shown that rotaviruses can survive for several months in fecal suspensions held at room temperature. Moe and Shirley (1982) investigated the survival of fecally suspended human rotavirus on experimentally-contaminated glass coverslips; under ambient conditions of temperature and relative humidity, the virus was found to retain its infectivity for several days. This indicates that surfaces and objects contaminated with rotavirus-positive material could act as a source of these viruses for prolonged periods. Keswick et al. (1983) were in fact able to recover infectious rotavirus particles from certain types of surfaces in a daycare centre.

The information summarized above indicates that water, food, and contaminated surfaces could act as vehicles in the spread of rotaviral diarrhea. However, there is some indirect evidence to suggest that spread of rotaviral infections may also occur by the airborne route. Kraft (1957) 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 (Kraft, 1958). Middleton et al. (1975) reported the spread of

human rotavirus infection from inoculated to uninoculated 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 the inhabitants of a group of islands in the mid-Pacific was shown to be due to a rotavirus with the help of retrospective serological studies (Foster et al., 1980). The high attack rate and the rapid spread of the outbreak were strikingly similar to those of influenza. On these grounds, it was suggested by these investigators that air may have acted as a vehicle in this outbreak.

A number of epidemiological studies on outbreaks of rotaviral diarrhea in certain temperate regions have noted a relationship between the occurrence of such outbreaks and the relatively low indoor RH (Brandt et al., 1983). Data available from tropical areas also indicate that an increase in the number of cases of rotaviral diarrhea in the general community often coincides with periods of cool and dry weather (DeTorres et al., 1978; Stintzing et al., 1981; Paul and Erinle, 1982).

In many patients during nosocomial outbreaks of rotaviral diarrhea, involvement of the respiratory tract precedes the diarrheal phase (Lewis et al., 1981). Attempts to detect rotaviruses in nasal swabs and washings have, however, been unsuccessful to date (Nigra and Midula, 1983). Here it should be noted that replication of rotaviruses in the respiratory tract may not be necessary before the production of gastroenteritis; virus-containing aerosols collected in the respiratory tract could be translocated by mucociliary activity and ingested (Slote, 1976). Intranasal instillation of rotavirus-containing inocula

has in fact been shown to result in diarrhea in piglets (Woode et al., 1976) and calves (Light and Hodes, 1949).

CORONAVIRUSES

Members of the family Coronaviridae form a single genus, Coronavirus. They are known to infect humans as well as a variety of animal species. On the basis of their characteristic virion morphology, they were first proposed as a taxonomic group in 1968 (Tyrrell et al., 1968). They have recently been defined more fully with the help of certain biological and molecular criteria. The virions of these viruses are enveloped and pleomorphic and range in particle diameter from 80-220 nm. They contain a single stranded RNA genome of positive polarity and bear widely spaced, club-shaped surface projections of about 20 nm in length (McIntosh, 1974; Robb and bond, 1979; Macnaughton and Davies, 1981; Wege et al., 1982; Siddell et al., 1982; 1983; Sturman and Holmes, 1983).

Seroepidemiologic studies suggest that human infections due to coronaviruses occur in all age groups and are distributed worldwide (McIntosh et al., 1974; Kaye et al., 1971, 1975; Gerna et al., 1978). They are known to cause respiratory infections in man and to date 3 different coronavirus strains (229E, OC43 and B814) have been isolated from humans.It has been estimated that they are responsible for 15-20% of colds in human adults (Larson et al., 1980; Monto, 1982). Results of virological and serological studies also indicate a possible association of coronaviruses with more severe diseases such as pneumonia, pleurodynia, myocarditis, meningitis (Riski and Hovi, 1980),

and degenerative diseases such as multiple sclersis (Robb and Bond, 1979).

In addition to the diseases mentioned above, human coronaviruses have been detected in enteric infections as well (Caul et al., 1975; Caul and Clarke, 1975; Moore et al., 1977; Caul and Egglestone, 1977; Schnagl et al., 1978; Moscovici et al., 1980; Caul and Egglestone, 1982). However, their role in causing gastroenteritis in man has not been clearly established. Available evidence suggests that they may cause diarrhea in psycho-geriatric patients (Clarke et al., 1979). A coronaviral etiology has also been suggested for neonatal necrotizing enterocolitis (Chany et al., 1982; Rousset et al., 1984) and the antigenic relationship of isolated human enteric coronaviruses to human coronavirus OC43 has been reported (Gerna et al., 1984). More recently (Rettig and Altshuler, 1985), coronavirus-like particles have been implicated in a fatal case of acute gastroenteritis of a 15-month old infant.

Cornaviruses have also been isolated from a variety of animal species, and in these hosts they have been found to cause a number of infectious diseases of economic importance (Clarke et al., 1979; Garwes, 1982). In pigs for example, they have been associated with outbreaks of acute diarrhea (transmissible gastroenteritis) (Pensaert and DeBouk, 1978) as well as central nervous system disorders (hemagglutinating encephalomyelitis of pigs) (Greig et al., 1962).

There appears to be a seasonal pattern to the occurrence of outbreaks of colds in humans and a variety of diseases in animals caused by coronaviruses. In humans, such outbreaks have been reported to occur in the late winter or early spring months in North America (McIn-

tosh et al., 1970; Cavallaro and Monto, 1970). However, no marked seasonal pattern for such infections was found in Great Britain (Macnaughton, 1982). In animals, cases of TGE in swine have been noted to occur between November and January, and decline from May through the summer (Ferris, 1973). A similar seasonal pattern has been noted in coronavirus infections of other animal species (Garwes, 1982).

While the mechanisms of spread of these agents in nature are not fully understood, air has been suspected as a vehicle for their direct or indirect dissemination in both humans and animals (Robb and Bond, 1979; Garwes, 1982; Monto, 1982). Intranasal instillation of coronavirus-containing inocula has been shown to produce disease in human volunteers (Reed, 1984) and pigs (Andries and Pensaert, 1981). In poultry, infection of the eyes through the aerosol route has also been shown with IBV (Cowen et al., 1971).

Despite this, very little is known about the survival of these viruses in the environment. The generally fastidious nature of coronaviruses and the difficulties in adapting them to grow in cell culture seriously hinder their isolation from clinical specimens, especially the putative enteric strains. A sensitive and reproducible plaque assay system for HCV/229E has been developed (Kennedy and Johnson-Lussenburg, 1975/76) and it has been demonstrated that HCV/229E can be recovered from experimental aerosols using LVAS (Park, 1980). The present study was designed, therefore, to investigate the capacity of these viruses to survive in the airborne state with particular reference to the effect of both RH and air temperature.

MAIN OBJECTIVES OF THE STUDY

The following were the main objectives of the present investigation.

1. To establish suitable procedures for the study of the airborne survival of rota- and coronaviruses.
2. To develop the use of poliovirus type 1 (Sabin) as a reference standard in the study of the aerobiology of rota- and coronaviruses.
3. To study the effect of various levels of relative humidity and air temperature on the airborne survival of laboraroty-adapted strains of rotaviruses of human and animal origin.
4. To aerosolize a laboratory-adapted strain of human rotavirus from a fecal suspension and study its survival in the airborne state.
5. To aerosolize field strains of human or animal rotaviruses contained in diarrheic feces and compare their airborne survival with that of the laboratory-adapted strains.
6. To study the effect of various levels of relative humidity and air temperature on the airborne survival of a laboratory-adapted strain of human coronavirus.
7. To discuss the findings with reference to the seasonality of outbreaks of infections due to rota- and coronaviruses.

MATERIALS AND METHODS

Cells:

MA-104 Cells: This line, derived from embryonic rhesus monkey (Macaca mulatta) kidneys (Microbiological Associates Inc., Bethesda, MD, U.S.A.) was used throughout for the cultivation and quantitation of rotaviruses. A seed culture of these cells was originally received by us through the courtesy of Dr. H. Malherbe (Gull Laboratories, Salt Lake City, UT, U.S.A.).

As stock cultures, monolayers of these cells were routinely cultivated in 75 cm^2 plastic culture flasks (Flow Laboratories, Rockville, MD, U.S.A.) using Eagle's minimal essential medium (MEM) in Earle's base (Autopow; Flow Labs.). Each 450 mL of the medium was supplemented with 25mg Garamycin (Schering Corporation, Kenilworth, NJ, U.S.A.), 13.5 mL of a 5.6% solution of sodium bicarbonate (J.T. Baker Chemical Company, Philipsburg, NJ, U.S.A.), 5.0 mL of a 200 mM solution of L-glutamine (Flow Labs.) and 50 mL of virus- and mycoplasma-tested fetal calf serum (FCS; Flow Labs.). Each monolayer was trypsinized using 1.0 mL of a mixture of 0.25% trypsin (Millipore Corporation, Bedford, MA, U.S.A.) and 0.05% ethylenediaminetetraacetic acid (EDTA) in Ca^{2+}- and Mg^{2+}- free phosphate buffered saline (PBS). A split ratio of 1:3 was generally used for the passage of the cells.

MA-104 cell cultures for plaque assay were put up in 12-well plastic plates (Costar, Cambridge, MA, U.S.A.). Each well in the plate was seeded with approximately 5.0×10^4 cells in 2.0 mL of the growth medium; the plates were then sealed individually in plastic bags (Philips Electronics Ltd., Toronto, Ont.) before being placed at 37°C in an ordinary walk-in incubator. The monolayers were generally ready for use in plaque assay within 48 hours of seeding.

L-132 Cells: These were originally derived from human embryonic lung tissue by Davis and Brolin (1970). This continuous line of human lung cells was used throughout this study to grow and quantitate the coronavirus. A seed culture of these cells was originally obtained from Mr. D. A. McLeod of the Laboratory Centre for Disease Control (L.C.D.C.), Ottawa, Ontario. The cells were maintained between passages 20 and 40 (from the date of introduction in our laboratory). As growth medium, MEM (Flow Labs.) was supplemented with 10% FCS (Flow Labs.), sodium bicarbonate (20mM), penicillin (100 u/mL), streptomycin (100 ug/mL), neomycin (50 ug/mL) and glutamine (2 mM). The L-132 cell monolayers were passaged every two days at a split ratio of 1:3. All cells were grown in 75 cm^2 disposable plastic flasks (Lux Scientific Inc.) and were used within 24 hours of reaching confluence.

Viruses:

Rotaviruses: Five laboratory-adapted strains of human and animal rotaviruses were used in this study. Simian rotavirus SA-11 (strain H-96) was supplied to us by Dr. Malherbe. Calf rotavirus (strain C-486) was obtained from Dr. L. A. Babiuk (University of Saskatchewan, Saskatoon, Sask.). A laboratory-adapted strain (Wyatt et al., 1980) of human rotavirus (D strain, subgroup 2, serotype Wa) was sent to us by Dr. R. G. Wyatt (National Institutes of Health, Bethesda, MD., U.S.A.). The mouse rotavirus was a gift from Dr. A. Cepica of the L.C.D.C., and the Compton U.K. strain of bovine rotavirus was obtained from Dr. M.A. McCrae (University of Warwick, Coventry, U.K.). All these viruses were first plaque purified by us once in MA-104 cells and the same cell line was used for preparing the virus pools.

Since FCS used for cell culture in this study was found to be inhibitory to the rotaviruses, it was necessary to wash the monolayers at least 3 times with Earle's balanced salt solution (EBSS; Flow Labs.) before virus inoculation. After allowing the virus under test to adsorb for one hour at 37°C, MEM without FCS and with 5.0 ug/mL of trypsin (1:250; ICN Nutritional Biochemical Company, Cleveland, OH, U.S.A.) was introduced into the cultures and they were placed back at 37°C. When nearly 75% of the monolayer was affected by rotavirus cytopathic effects, the cultures were frozen (at -80°C) and thawed 3 times. The cell culture harvest was centrifuged for 15 minutes at 1000 x g in a DPR-6000 centrifuge (Damon, IEC Division, Fisher Scientific Ltd., Pittsburg, PA, U.S.A.). The supernatant was extracted with an equal volume of 1,1,2-trichloro-1,2,2-trifluoroethane (Freon; J.T. Baker Chemical Co.). The aqueous (top) phase was then removed and centrifuged at 100,000 x g for 120 minutes in a Beckman LS-250 centrifuge (Beckman Instruments Inc., Toronto, Ont.).

The pelleted virus was resuspended in tryptose phosphate broth (TPB) and then passed through a membrane filter (Millipore Corp., Bedford, MA, U.S.A.) with a pore diameter of 0.45 um in order to remove larger particulate matter. To ensure that the pools contained only monodispersed virus particles, they were subsequently filtered using polycarbonate membranes (Nuclepore Corp., Pleasanton, CA, U.S.A.) with a pore diameter of 80 nm.

Coronavirus: Human coronavirus strain 229E (HCV/229E) was originally obtained by us from Dr. A. Z. Kapikian of the U.S. National Institutes of Health. The virus was cultivated in 75 cm^2 monolayers of

L-132 cells using MEM with 2% FCS. After incubation at 33°C for 36 hours, the virus was released from the cells by freezing and thawing the flasks and contents three times. The suspension was transferred to centrifuge tubes and clarified by centrifugation at 2000 rpm for 15 minutes at 4°C. Each nine mL of the virus-containing supernatant fluid was mixed with 1.0 mL of 10X TPB and then stored at -80°C.

Poliovirus: The Sabin strain of poliovirus type 1 was kindly provided to us by Mr. McLeod of the L.C.D.C. The pools of this virus were also prepared in either MA-104 cells or L-132 cells. When this virus was to be mixed with rotaviruses, its pools were prepared in MA-104 cells and concentrated in a similar way to those of the rotaviruses except that in the final stage they were filtered through polycarbonate membrane filters with a pore diameter of 30 nm. In experiments with mixtures of polio- and coronavirus, it was grown in L-132 cells and processed in a similar way to HCV/229E.

Radiolabelling of Rotavirus SA-11:

Two-day old confluent monolayers of MA-104 cells were incubated for 18 hours at 37°C in methionine-free MEM. They were then washed 3 times with EBSS and inoculated with the virus at a multiplicity of infection of 5:1. The virus was allowed to adsorb to the cells for 2 hours at 37°C. The labelling was carried out using methionine-free MEM with 50 uCi/mL of L-(^{75}Se) selenomethionine purchased from Amersham Corp. (Arlington Heights, IL, U.S.A.). The cultures were held at 37°C for 24 hours. They were then put through the same procedure as described above for the preparation of the virus pools. However, after ultracentrifugation, the pellet of the labelled virus was resuspended in a 40% (w/v) solution of cesium chloride (CsCl) and layered onto a

55% solution of CsCl and then subjected to isopycnic banding by centrifugation at 100,000 x g for 20 hours. The fractions were collected from the bottom of the centrifuge tube and their refractive index measured. Those fractions with the density range of 1.36-1.39/mL were pooled together and dialysed against distilled water overnight at 4°C. The dialysed virus was resuspended in TPB and stored at -80°C until used.

Poliovirus Antiserum:

Hyperimmune rabbit antiserum against the poliovirus was kindly provided to us by Dr. P. Payment (Institute Armand-Frappier, Ville de Laval, Quebec). This antiserum, when added to a polio-rotavirus or polio-HCV/229E mixture at a final concentration of 1:1000, completely inhibited poliovirus plaques without affecting the number or appearance of either the rotavirus or coronavirus plaques.

Plaque Assays:

Rotaviruses: The quantitation of rotavirus plaque forming units (PFU) was carried out using the technique of Ramia and Sattar (1979a). Monolayers of MA-104 cells in 12-well plates were thoroughly washed with EBSS to remove all traces of FCS present in the culture medium. Each well then received 0.1 mL of the desired inoculum and the cultures were incubated at 37°C for 1 hour on a pitched orbital shaker. Each one of the control wells received 0.1 mL of EBSS. The overlay medium (2mL/well) consisted of MEM, 0.6% Agarose Type II (Sigma Chemical Company, St. Louis, MO, U.S.A.) and 5.0 ug/mL of trypsin (Nutritional Biochemical Co.). The plates were resealed in the plastic bags and placed in a walk-in incubator at 37°C.

At the end of 3 days of incubation, the cultures were fixed over-

night in a 10% formalin (J.T. Baker Chemical Co.) solution in normal saline. After the removal of the fixative and the overlay medium, the monolayers were washed with tap water and then stained with a 1% aqueous solution of crystal violet.

Coronavirus: Titrations of this virus were performed by plaque assay in monolayers of L-132 cells in 75 cm^2 disposable culture flasks (Lux Scientific Corp.) as previously described (Kennedy and Johnson-Lussenburg, 1975-76). Cell monolayers generally less than 48 hours old were used. The growth medium was removed and the monolayers were washed with PBS three times before they were infected with an appropriate dilution of the virus. The PBS used in the last wash was allowed to remain on the monolayers until they were infected. Virus dilutions were made in Medium-199 (M199) in Earle's base (Flow). After removing the PBS, each monolayer was inoculated with 0.33 mL of the virus sample.

The virus was adsorbed for one hour at room temperature on a pitched orbital shaker. At the end of the incubation, each monolayer was overlayed with 25 mL of a medium containing M-199, 0.6% (final conc.) Oxoid Agar NO. 1, 21.4 mL of 5.6% sodium bicarbonate, 3.0 mL of 1.0% 5-bromodeoxyuridine (BUDR, Calbiochem), and 3.0 mL of 4.0% DEAE Dextran (Sigma). The overlay medium also contained penicillin, streptomycin and neomycin at final concentrations of 100 units/mL, 100ug/mL and 50 ug/mL, respectively. For plaque development, the inoculated cultures were incubated, upside down, at 33°C for 6 days.

Following incubation, 5.0 mL of 4% formalin in saline was added to each of the flasks which were then incubated at 37°C for 1 hour or allowed to stand at room temperature overnight to inactivate the virus and fix the cells. The agar overlay was then carefully removed and the

monolayer was stained with a crystal violet solution as described above.

Poliovirus: When titrated in MA-104 cells, the procedure for the plaque assay of the poliovirus was identical to that of the rotaviruses, except that the overlay medium for the poliovirus did not contain any trypsin. The procedure for the plaque assay of this virus in L-132 cells was identical to that used for the coronavirus. In either cell line, the plaques of this virus were generally ready to be stained and counted within 48 hours of incubation at 37°C.

Suspending Media:

In this investigation, TPB was selected as the medium for the generation and collection of viral aerosols. Earlier work carried out in our laboratory (Ramia and Sattar, 1980) had revealed it to be harmless for rotaviruses. Preliminary tests carried out as a part of this study also showed to be safe for the infectivity of polio- and coronaviruses. Moreover, as opposed to the use of MEM, TPB was considered to simulate more closely the composition of body secretions and excretions.

In order to further test the validity of using TPB as a suspending medium for rotaviruses, a limited number of experiments was conducted where the cell culture adapted strain of human rotavirus was aerosolized from a fecal slurry (10% W/V) obtained from a laboratory-confirmed case of rotavirus diarrhea. The fecal sample was thoroughly secreened for the presence of viruses which could produce cytopathology or readily visible plaques in MA-104 cells. Results of such testing revealed that the presence of fecal suspension was not producing any

alteration in the plaque titration of the cell culture-adapted strain of human rotavirus.

Nebulizer:

The viral aerosols generated in the present study were produced with the help of a 6-jet Collison nebulizer (May, 1973) purchased from BGI Incorporated (Waltham, MA, U.S.A.). Between experiments, the nebulizer was cleaned by sonication in a detergent solution and sterilized by autoclaving.

Rotating Drum:

The chamber used for holding the viral aerosols was a 300-L cylindrical stainless steel drum (Goldberg, 1958). It was housed in an insulated wooden cabinet. A refrigeration unit inside the cabinet could be used to maintain the temperature inside it at the desired level. For all the experiments reported here, the drum was rotated at the rate of four rpm.

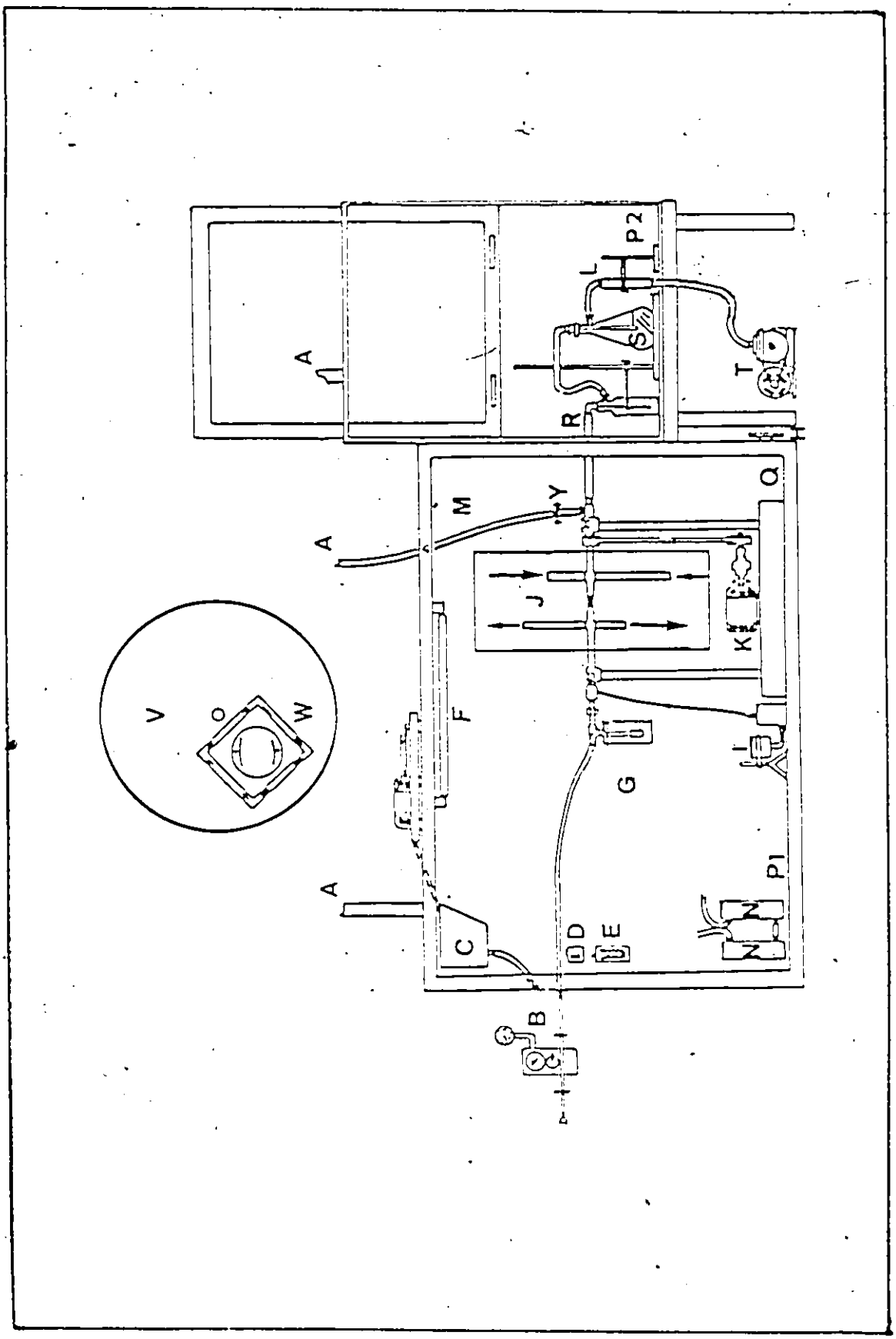
The drum and the cabinet were custom made nearly 25 years ago according to the specifications provided by the late Dr. C. A. Mitchell of this department (Guerin and Mitchell, 1964). The cabinet with the drum is presently installed in a level 'C' (Medical Research Council of Canada, 1980) biohazards containment facility in our Faculty (Figure 2).

Impinger:

All-glass impingers (AGI) were used throughout this study to collect the viral aerosols from the air in the rotating drum. They were purchased from BGI Incorporated and modified in our laboratory to give a distance of 15 mm between the tip of the limiting orifice and the surface of the collecting fluid (10 mL) in the impinger. When

Figure 2. Schematic diagram of the equipment used for the generation, storage and collection of virus aerosols.

A = exhausted air to HEPA filter in ceiling; B = pressure regulator for compressed air being supplied to the nebulizer; C = refrigeration unit; D = thermostat; E = thermometer; F = ultraviolet light; G = Collison nebulizer; H = air inlet filter; I = Drierite tank; J = rotating drum; K = motor; L = Drierite container; M = air overflow from rotating drum; N = Drierite; P^1 = insulated containment cabinet; P^2 = fume hood; Q = cabinet air inlet filter; R = all-glass impinger; S = liquid disinfectant; T = vacuum pump; V = side view of rotating drum; W = porthole with inserted hygrometer; Y = clamp to close circuit.


A
P2
L
S
T
R
Q
M
Y
A
J
K
V
O
W
F
G
I
A
P1
C
D
E
N
B

collecting the air samples, a critical vacuum was maintained to operate the impinger at its design capacity of 5.6 L/min. Between experiments, the impinger was cleaned by sonication in a detergent solution and sterilized by autoclaving.

Adjustment of Relative Humidity and Temperature:

The effect of the following 3 levels of relative humidity (RH) on the survival of airborne viruses was tested in this study: low (30±5%), medium (50±5%) and high (80±5%). All RH measurements were made with the help of a dial-type (15 cm in diameter) hygrometer (Airguide Instruments Co., Chicago, IL, U.S.A.) affixed to the drum. The accuracy of this meter was periodically checked using a calibrated Heathkit electronic hygrothermometer. The procedures for achieving the desired RH levels of the air in the drum were as follows:

For the experiments at the low RH, the drum was first filled with air passed through a Drierite cylinder (Hammond Drierite Co., Xenia, OH, U.S.A.) prior to nebulization of the virus suspension. This generally brought the RH level inside the drum to 15-20%. In order to achieve an RH of about 30%, the nebulizer containing the virus suspension was operated only for a 1-minute period which contributed enough moisture to bring the RH up to the desired level. To achieve the medium and high RH levels, distilled water was sprayed into the drum until the RH level in the drum was 35-40% and 65-70%, respectively. Following a short equilibration period, the test virus was aerosolized in to the drum by operating the nebulizer for 5 min for the medium RH and 10 min for high RH. Once again, moisture added during nebulization brought RH up to the desired level.

The temperature of the air inside the drum was maintained at

either 20±1°C or 6±1°C by means of the thermostatically controlled refrigeration unit housed within the drum cabinet (Figure 2).

Fluorescent Dyes as Physical Tracers:

Either rhodamine B (Kodak, Rochester, NY, U.S.A.), or uranine (sodium fluorescein; Fisher Scientific Co., Fair Lawn, NY, U.S.A.), was used as a physical tracer (Robinson et al., 1959; Beard and Easterday, 1965; Songer, 1967; Elazhary and Derbyshire, 1979a) in this study. The final concentrations of rhodamine and uranine in the spray fluid were 2.5 mg/mL and 1.0 mg/mL, respectively. Reference solutions for the standardization of the dye were prepared in TPB or MEM. An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Springs, MD, U.S.A.) was used for measuring the dye concentration in the samples. The excitation and emission wavelengths used were 546 and 590 nm (rhodamine B) and 493.5 and 512 nm (uranine), respectively. This instrument was found to be capable of detecting the dyes at concentrations as low as 0.1 parts per billion (Udenfriend, 1964). To avoid any variations in dye measurements due to sample temperature, all samples were brought to room temperature before placing them in the machine.

In order to determine the suitability of the fluorescent dyes as physical tracers in this study, initial comparative tests were performed using ^{75}Se-labelled rotavirus SA-11. All radioactivity measurements were carried out using a scintillation counter (Model LS 250, Beckman Instruments Inc., CA, U.S.A.).

Antifoam:

Presence of proteins in the spray and collection fluids led to excessive frothing during the nebulization and collection of the aem-

ples. Either autoclave sterilized Antifoam A (Sigma) or Antifoam C (Sigma) was routinely added to the spray or collection fluid in order to reduce such frothing. The final concentrations of Antifoam A and Antifoam C in the above mentioned fluids were 0.001% and 1.0%, respectively.

Determination of the Size Range of the Aerosols:

The size range of the virus aerosols in the drum air was determined at three RH levels using the Anderson six-stage stacked-sieve sampler (Anderson, 1958). Each glass petri dish specifically designed for use with this sampler contained 27.0 mL of a semi-solid medium prepared as follows using a modification of the formula of Guerin and Mitchell (1964):

A 10% (w/v) solution of gelatin (Fisher Scientific Co., NJ, U.S.A.) was prepared in sterile distilled water and heated at 100°C for about 30 min. Autoclave sterilized 1X TPB was brought to a temperature of 37°C and the gelatin solution was added to it to give a final concentration of 3%. The plates containing the aerosol collection medium were sealed with parafilm and were placed at 4°C until used.

Immediately prior to the collection of the air sample, the plates were placed in the six stages of the Anderson sampler. The inlet of the sampler was then attached to the outlet of the rotating drum with the help of a rubber tube and the sampler was operated for 1 minute to draw 28.0 L of the air. The air samples from the drum were collected at 0.25, 2 and 8 hours of aerosol age. The same Anderson sampler and vacuum pump (supplied with the sampler) were used throughout these experiments in order to avoid any variations due to these pieces of equipment.

After the sample was collected, the petri plates were incubated at 37°C to melt the gelatin in the collection medium. The melted medium from each plate was poured separately into screw-capped glass vials and used for the measurement of the concentration of the physical tracer as well as the virus PFU.

Test Procedure:

Several hours before the start of the experiment, the temperature and RH of the air inside the rotating drum were adjusted to the desired level. They were periodically monitored during this period to ensure their stability prior to the spraying of the virus under test.

When ^{75}Se-labelled virus was to be nebulized, it was suspended in TPB alone. However, the spray fluid for the unlabelled virus also contained the appropriate dye and antifoam. The Collison nebulizer, with 10 mL of the spray fluid, was attached to the inlet of the rotating drum and aerosolization was carried out for the required length of time at a pressure of 1.8 kg/ cm^2 using compressed air from a central supply in the building. The volume of the fluid sprayed was determined by weighing the Collison nebulizer before and immediately after aerosolization. Samples of spray fluid were also withdrawn immediately before and after nebulization to determine the amount of virus and dye sprayed. These data were used to calculate the quantity of the 'input virus' into the drum.

The first air sample from the drum was collected with the impinger at the end of the 15 min period of aerosol stabilization. This was done by operating the impinger for 2 min to withdraw 11.2 L of the air sample. Depending on the duration of the test run, additional aerosol

samples were collected at regular intervals. In order avoid excessive dilution of the aerosols with make-up air during sampling, no more than a total of 5 samples were taken over the entire time course of an experiment. The same nebulizer and impinger were used throughout this study in order to avoid variations between samples and experiments.

After collection, the fluid from the impinger was divided into 2 portions. One of these was used for estimating the amount of the physical tracer, and the other, after passing it through a 0.22 um membrane filter, was used for virus plaque assay. The extent of biological decay of the virus in air was calculated using the following formula:

$$\% \text{ VIRUS SURVIVAL} = (D_o / D_t) \times (V_t / V_o) \times 100$$

where D_0 and D_t are the tracer concentrations at time 0 and time t, respectively, and V_0 and V_t are the virus titres at time 0 and t, respectively. It should again be noted here that the time 0 sample is the one withdrawn immediately at the end of the 15 min period of aerosol stabilization in the drum. The difference between the 'input virus' concentration and that in the first air sample was regarded as the 'initial loss' in the infectivity of the aerosolized virus.

The survival of the aerosolized virus under various experimental conditions was plotted against time using an exponential model,

$$\% \text{ virus survival} = A \times \exp(-B \times \text{Time})$$

and the coefficients A and B were estimated by least squares curve fitting. At least three experiments at each RH and temperature were carried out and the half-lives of the viruses were calculated by regression analysis (Lawless, 1982).

EXPERIMENTAL RESULTS

(1) Methodology Development:

In order to be able to compare the data generated on the airborne survival of the various types of viruses tested, it was essential to keep the variations in materials and techniques to a minimum. In view of this, during the initial stages of this study, a number of materials and techniques were tested for their suitability in working with all the viruses selected here. This section of 'Experimental and Results', therefore, describes the basis of such testing and gives the reasons for choosing a particular type of material or technique for the subsequent studies on the aerobiology of rota-, corona- and polioviruses. As has been mentioned earlier, the fact that a rotating drum for the aging of viral aerosols was already available in this department greatly facilitated the experimentation. Following are the details of the experimental design and the results obtained:

Physical Tracers and Antifoams: In studies on the airborne survival of viruses it is essential to account for the dilution of the aerosolized virus in the air and physical loss of the sprayed material due to sedimentation before the rate of biological decay of the virus can be determined (Spendlove and Fannin, 1982). This is generally achieved by adding a 'physical tracer' to the virus suspension to be aerosolized. Substances such as fluorescent dyes (Beard and Easterday, 1965; Songer, 1967; de Jong et al., 1973; Elazhary and Derbyshire, 1977), radioisotopes (Wolfe, 1961; Harper, 1961; May, 1973; Trouwborst and deJong, 1973) and bacterial spores (Rabey et al., 1969; Benbough, 1969; deJong and Winkler, 1964; Ehrlich and Miller, 1972; Donaldson and Ferris, 1976) have been employed for this purpose.

Radioisotopes incorporated into virus particles through labelling

of either viral protein or nucleic acid are considered to be highly desirable as physical tracers. There are, however, the disadvantages of the high cost and potential health hazards involved in the regular use of such labelled preparations. On the other hand, fluorescent dyes such as rhodamine B and uranine are relatively inexpensive and safe for use as physical tracers. But before any one of these two substances could be used in this study, it was considered essential to determine that their presence in the spray fluid would not be deleterious to the infectivity of the viruses and their quantitation.

A great deal of froth was generated when TPB was used as the virus spray and aerosol collection fluid. It was, therefore, necessary to suppress this frothing by the use of an antifoam which would not inactivate the viruses or in some way interfere with virus plaque assay.

A known amount of rotavirus SA-11, which was the virus used in the initial phases of this study, was suspended in TPB with and without the dyes and the antifoams. The suspensions were held at room temperature for 30 min before being plaque assayed. The results of these tests are presented in Table 2. Rhodamine B and Antifoam C, alone or in combination with each other, reduced the virus plaque titre by at least 4-fold. On the other hand, neither uranine nor Antifoam A, separately or as a mixture, were found to affect the infectivity of the virus.

Subsequent tests showed (Table 3) that uranine and Antifoam A were equally harmless to the infectivity of the corona-, polio- and other rotaviruses used in this study. In view of this, these two substances were routinely added to the virus spray fluid. Antifoam A was also regularly incorporated in the aerosol collection fluid.

TABLE 2

EFFECT OF THE PHYSICAL TRACERS AND ANTIFOAMS ON THE INFECTIVITY OF SIMIAN ROTAVIRUS SA-11

Suspending medium	Virus PFU/mL x 10^7
Tryptose phosphate broth (TPB)	1.70
TPB with Antifoam C	0.30
TPB with Rhodamine B	0.40
TPB with Rhodamine B and Antifoam C	0.40
TPB	6.50
TPB with Uranine	6.52
TPB with Antifoam A	6.49
TPB with Uranine and Antifoam A	6.51

The virus was added to TPB alone or TPB containing the additive(s) under test. After an incubation of 30 min at room temperature (22°C), the samples were diluted in EBSS and plaque assayed.

TABLE 3

EFFECT OF URANINE AND ANTIFOAM A ON THE INFECTIVITY OF CORONA, POLIO AND ROTAVIRUSES

Viruses	Virus PFU/mL X 10^7			
	TPB alone	TPB with Uranine	TPB with Antifoam A	TPB with Uranine & Antifoam A
Corona 229E	2.10	2.13	2.18	2.22
Polio 1(Sabin)	5.00	4.50	4.80	4.70
Rota (Wa)	6.00	6.10	5.80	5.90
Calf Rota (C-486)	1.40	1.30	1.40	1.50
Calf Rota (UK)	70.00	69.50	71.00	70.50
Mouse Rota	45.00	46.00	45.00	45.50

The virus under test was added to TPB alone or TPB containing the additive(s) under test. After incubation of 30 min at room temperature (22°C), the samples were plaque assayed.

Effect of the Process of Nebulization on the Viability of the Viruses: During the operation of the Collison nebulizer, virus particles present in the spray fluid are subjected to strong shearing forces. This could lead to the inactivation of the virus being aerosolized. In order to test this, the titre of the virus in the spray fluid was measured before and after operating the nebulizer for 10 min. There was no significant loss in the viability of the corona-, polio- and rota-viruses tested (Table 4). These findings established the fact that the Collison nebulizer could be safely used for the aerosolization of all the viruses selected for this study.

Effect of Nebulization on the Concentration of the Dye in the Spray Fluid: In an earlier study, it was suggested that passage of dry air through a dye-containing spray fluid during the process of nebulization could result in the rapid evaporation of water and thus lead to an increase in the concentration of the dye in the spray fluid (Beard and Easterday, 1965). In order to test this, the concentration of the dye in the spray fluid (TPB) was measured before and after the operation of the nebulizer for 10 min. As can be seen from the data presented in Table 5, there was no detectable change observed in the concentration of uranine in the spray fluid as a result of the process of nebulization.

Suitability of Uranine as a Physical Tracer: Before any experiments on the airborne survival of the viruses could be carried out, it was considered imperative to determine the suitability of uranine as a physical tracer. In order to do this, uranine and Antifoam A were added to TPB containing ^{75}Se-labelled rotavirus SA-11. This mixture was aerosolized into the rotating drum (50±5% RH; 20±1°C) and samples of

TABLE 4

EFFECT OF NEBULIZATION ON THE VIABILITY OF CORONA, POLIO AND ROTAVIRUSES

Viruses	Virus PFU/mL X 10^7		% PFU
	Before Nebulization	After Nebulization	Recovered
Corona 229E	1.45±0.57	1.41±0.58	98.80±3.90
Polio 1(Sabin)	5.00±0.92	5.10±1.00	101.0±2.40
Rota (Wa)	4.45±2.00	5.00±2.60	111.8±14.9
Calf Rota (C-486)	2.40±1.47	2.41±1.26	106.4±16.4
SA-11	3.45±1.56	3.50±1.6	101.45±5.34
Calf Rota (UK)	30.0*	31.0	103.33
Mouse Rota	63.0*	62.5	99.21

A 10 mL volume of TPB containing the virus under test was added to the Collison nebulizer which was then operated for 10 min. The virus was plaque assayed to determine its titre in the suspending medium before and after the nebulizer was operated. At least 4 experiments were performed and the figures represent the mean ± S.D.

* No S.D. available because only one experiment was carried.

TABLE 5

EFFECT OF NEBULIZATION ON THE CONCENTRATION OF THE PHYSICAL TRACERS IN THE SPRAY FLUID

Type of Dye	Dye Concentration in mg/mL		% Dye Recovered
	Before Nebulization	After Nebulization	
Rhodamine B	2.50 ± 0.02	2.49 ± 0.03	100.17 ± 0.67
Uranine	1.00 ± 0.01	0.99 ± 0.03	99.950 ± 0.10

A 10 mL volume of TPB containing either of these two dyes was added to the Collison nebulizer which was then operated for 10 min. The concentration was of the dyes was measured in an Aminco Bowman spectrophotofluorometer. At least four experiments were performed with each dye under test and the figures are expressed as mean ± S.D.

the air from the drum were collected at appropriate intervals and assayed for the dye, radioactivity and infectious virus. The results are presented in Figure 3. The rates of biological decay of the virus calculated using the two different physical tracers were found to be almost the same. These data clearly demonstrated the fact that a fluorescent dye could be not only relatively simple and safe, but also highly reliable as a physical tracer.

Determination of the Extent of Virus Inactivation During the Period of Aerosol Stabilization: Apart from the physical loss of the virus, the extent of virus inactivation immediately after its aerosolization depends largely upon the RH and temperature of the aerosol-receiving air. In aerobiology, such a reduction in virus infectivity is generally referred to as the 'initial loss'. That proportion of the airborne virus which survives the initial loss requires a certain amount of time for its uniform distribution in the air inside the rotating drum. In view of this, the first air sample from the drum was always collected 15 min after the termination of virus aerosolization. This period of aerosol stabilization was, therefore, considered essential to allow for (a) the initial loss in virus infectivity to occur, (b) the physical loss of larger particles to take place and (c) the homogeneous distribution of the airborne particles in the air inside the drum.

To determine the initial loss in virus infectivity, the amount of virus sprayed into the drum air under different conditions of RH and temperature was compared with the amount recovered in the first air sample. The results of these experiments are presented in Table 6.

At the low and medium RH levels, with an air temperature of

Figure 3. Comparison of the rates of biological decay of airborne rotavirus SA-11 at the medium relative humidity level (20±1°C) as determined with the use of two different physical tracers.

^{75}Se-labelled virus ○

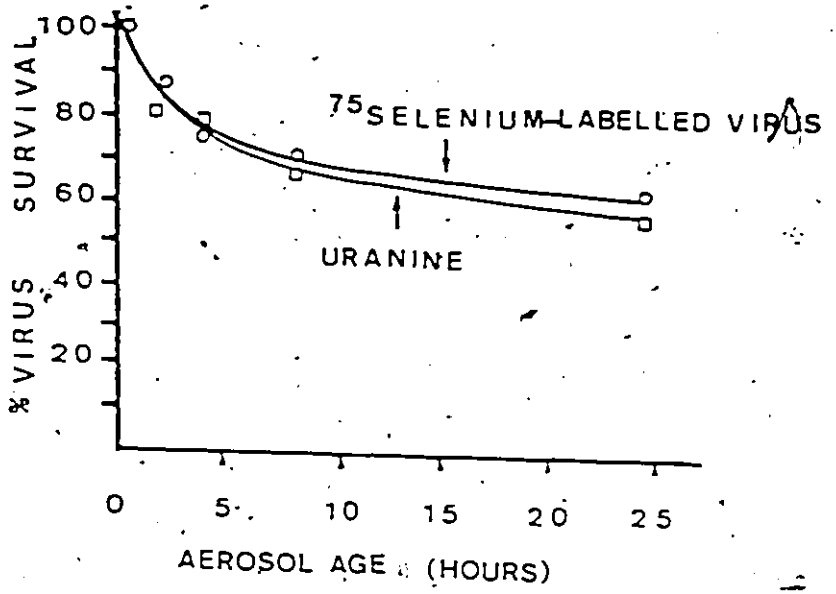
Uranine □

Figure 4. The effect of three different levels of relative humidity on the airborne survival of rotavirus SA-11 at 20±1°C.

RH 30±5% ●

RH 50±5% □

RH 80±5% ○


100
80
60
40
20
% VIRUS SURVIVAL
^{75}SELENIUM-LABELLED VIRUS
URANINE
0
5
10
15
20
25
AEROSOL AGE (HOURS)

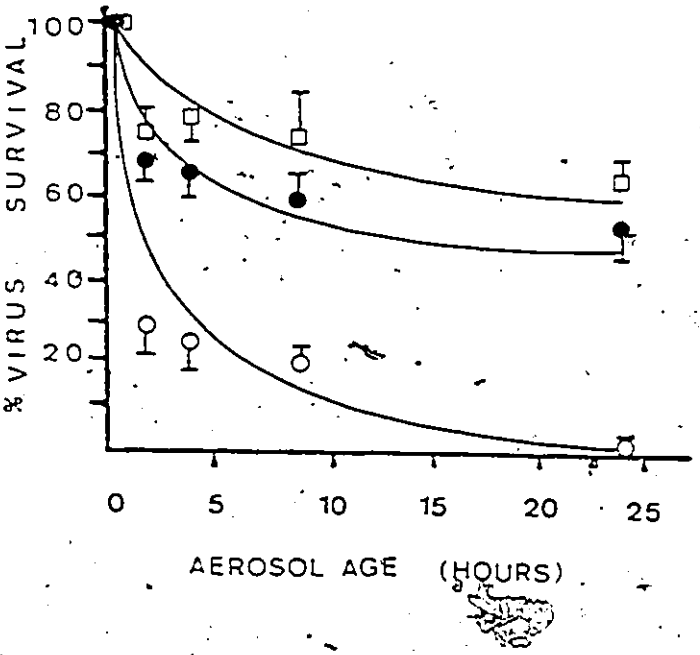

100
80
60
40
20
% VIRUS SURVIVAL
0
5
10
15
20
25
AEROSOL AGE (HOURS)

TABLE 6

PERCENT RECOVERY OF THE VIRUSES AFTER AEROSOLIZATION AND EQUILIBRATION OF THE AEROSOL CLOUD AT 20 ± 1°C

Viruses	Relative Humidity		
	High (80±5%)	Mid (50±5%)	Low (30±5%)
Corona 229E	55.0±3.5	90.9±1.6	87.0±2.5
Polio 1(Sabin)	104.1±7.8	0	0
Rota (Wa)	34.3±3.8	86.8±4.7	45.8±3.7
Calf Rota (C-486)	27.3±1.8	70.0±1.5	36.5±5.2
SA-11	24.7±4.7	78.0±2.3	42.1±2.6
Calf Rota (UK)	29.7	73.4	38.5
Mouse Rota	20.4	90.6	49.0

In all experiments a 15-minute period was allowed for aerosol stabilization and the first air sample was collected at the end of this period. To determine the extent of virus survival during this period, the titre of the virus in the air sample was compared with the amount of virus aerosolized. At least 3 separate experiments were conducted with all viruses except calf rota (UK)- and mouse rotaviruses. The results are presented as the mean ± S.D.

20±1°C, the initial loss in the infectivity of the poliovirus was so pronounced that no infectious virus could be detected in the air samples collected at the end of the aerosol stabilization period. In contrast to this, there was no detectable initial loss in the infectivity of the aerosolized poliovirus when the RH of the air inside the drum was kept at the high level with the air temperature at 20±1°C.

At 20±1°C, the initial loss in the infectivity of the 5 types of rotaviruses tested was the highest at 80±5% RH and they appeared to survive best during the aerosol stabilization period when the RH was at the medium level. Although lowering of the air temperature to 6±1°C showed an increase in the overall capacity of the human rotavirus to survive during the stabilization period, the initial loss in the virus titre was still the lowest at the medium RH level and greatest at the high RH level (Table 7).

At the higher air temperature, the general pattern in the initial loss of the coronavirus was very similar to that seen with the rotaviruses. However, when the air temperature was reduced to 6±1°C, not only was there a general reduction in the initial loss, but the virus appeared to survive equally well at all 3 RH levels (Table 7).

Determination of the Particle Size Distribution of Viral Aerosols Produced by the Collison Nebulizer and held at the 3 RH Levels (20±1°C): These experiments were performed to determine the size distribution of the virus-containing particles within an aerosol produced by the Collison nebulizer and stored in the rotating drum at the 3 levels of RH (20±1°C). Rotavirus SA-11 was suspended in TPB containing the dye and the antifoam. The virus suspension was sprayed into the drum and the viral aerosols held at the desired RH level. At 0.25, 2

TABLE 7

PERCENT RECOVERY OF THE VIRUSES AFTER AEROSOLIZATION AND EQUILIBRATION OF THE AEROSOL CLOUD AT 6 ± 1°C

Viruses	Relative Humidity		
	High (80±5%)	Mid (50±5%)	Low (30±5%)
Corona 229E	104.8±5.1	96.5±3.0	91.0±2.6
Rota (Wa)	43.8±3.3	95.8±2.6	57.2±3.8

After allowing a period of 15 minutes for aerosol stabilization, the first air sample was collected. To determine the extent of virus survival during this period, the titre of the virus in the air sample was compared with the amount of virus aerosolized. At least 3 seprate experiments were conducted and the results are presented as the mean ± S.D.

and 8 hours after virus aerosolization, samples of the air from the drum were collected with the help of an Anderson sampler (Anderson, 1958) according to the procedure described in 'Materials and Methods'.

The gelatin-containing medium from each of the six plates in the sampler was collected separately after incubating the plates for 1 hour at 37°C. These samples were then assayed for the dye as well as the infectious virus.

The results of these experiments are shown in Tables 8-10. Irrespective of the RH level, >87% of the infectious virus as well as the dye recovered from the drum air were collected in stages 4, 5 and 6 of the sampler. These 3 stages of the Anderson sampler are designed to recover airborne particles in the size range of <1.0-3.3 um in diameter (Anderson, 1958). Such particles can remain airborne for prolonged periods and, upon inhalation, they also have the potential for retention in the human respiratory tract.

Virus Mixtures: Because a considerable amount of information is available regarding the airborne survival of polioviruses (Harper, 1961; 1963; de Jong 1970; de Jong et al., 1973; Ijaz et al., 1984; 1985), the Sabin strain of poliovirus type 1 was used as a reference standard in our experiments dealing with the aerobiology of rota- and coronaviruses. Poliovirus was mixed with either a rotavirus or coronavirus and the mixture aerosolized. This allowed us to compare the rate of biological decay of both the viruses in the mixture under identical experimental conditions.

The air samples collected in these experiments contained poliovirus with either rota- or coronaviruses. Therefore, it was necessary to develop procedures so that the quantitation of one type of

TABLE 8

SIZE DISTRIBUTION OF DYE AND ROTAVIRUS SA-11 CONTAINING AIRBORNE PARTICLES AT RH 30±5% (20±1°C)

Aerosol Age (Hours)	Stage No.*	% Recovery		Cumulative % Recovery from last three Stages*	
		Dye	Virus	Dye	Virus
0.25	1	0.00	0.00		
	2	0.50	0.30		
	3	7.00	7.00		
	4	19.00	22.00		
	5	51.00	50.30	91.0	92.4
	6	18.00	20.4		
2	1	0.00	0.00		
	2	2.50	2.30		
	3	5.00	6.00		
	4	30.50	30.50		
	5	49.00	49.00	92.5	91.7
	6	13.00	12.20		
8	1	0.00	0.00		
	2	0.70	0.30		
	3	1.30	1.70		
	4	35.00	35.50		
	5	49.00	48.00	98.0	98.0
	6	14.00	14.50		
24	1	0.00	0.00		
	2	0.30	0.30		
	3	1.70	2.00		
	4	33.00	32.80		
	5	59.00	58.50	98.0	97.7
	6	6.00	6.50		

A 10 mL volume of TPB containing the virus and uranine (1mg/mL) was added to the Collison nebulizer and aerosolized into the rotating drum. After 15 minutes of cloud stabilization, a 1-min air sample (28 L) was drawn with the help of the Anderson sampler. Samples from each of the stages of the sampler were divided into two portions. The virus was quantitated by plaque assay and the concentration of the dye was measured by spectophotofluorometry.

*Stages No. 4, 5 and 6 are designed to recover particles with diameters of 2.0-3.3um, 1.0-2.0um and <1.0um, respectively.

TABLE 9

SIZE DISTRIBUTION OF DYE AND ROTAVIRUS SA-11 CONTAINING AIRBORNE PARTICLES AT RH 50±5% (20±1°C)

Aerosol Age (Hours)	Stage No.*	% Recovery		Cumulative % Recovery from last three Stages*	
		Dye	Virus	Dye	Virus
0.25	1	0.50	1.00		
	2	4.30	3.50		
	3	6.00	6.00		
	4	46.00	45.00		
	5	38.00	35.00	89.4	89.5
	6	5.40	9.5		
2	1	0.50	0.10		
	2	2.50	1.80		
	3	7.00	8.00		
	4	32.30	32.00		
	5	52.00	52.00	89.8	90.0
	6	5.50	6.00		
8	1	0.70	0.00		
	2	0.90	1.00		
	3	6.00	6.00		
	4	37.40	35.00		
	5	48.90	50.00	92.4	93.0
	6	6.10	8.00		
24	1	0.00	0.00		
	2	0.00	0.00		
	3	2.40	3.00		
	4	29.40	29.00		
	5	62.00	61.00	97.6	97.0
	6	6.20	7.00		

A 10 mL volume of TPB containing the virus and uranine (1mg/mL) was added to the Collison nebulizer and aerosolized into the rotating drum. After 15 minutes of cloud stabilization, a 1-min air sample (28 L) was drawn with the help of the Anderson sampler. Samples from each of the stages of the sampler were divided into two portions. The virus was quantitated by plaque assay and the concentration of the dye was measured by spectophotofluorometry.

*Stages No. 4, 5 and 6 are designed to recover particles with diameters of 2.0-3.3um, 1.0-2.0um and <1.0um, respectively.

TABLE 10

SIZE DISTRIBUTION OF DYE AND ROTAVIRUS SA-11 CONTAINING AIRBORNE PARTICLES AT RH 80±5% (20±1°C)

Aerosol Age (Hours)	Stage No.*	% Recovery		Cumulative % recovery from last three stages*	
		Dye	Virus	Dye	Virus
0.25	1	0.45	0.50		
	2	0.49	0.40		
	3	0.90	1.00		
	4	28.00	30.10		
	5	49.25	48.00	98.25	98.10
	6	21.00	20.00		
2	1	0.40	0.50		
	2	2.40	2.00		
	3	5.00	4.00		
	4	52.00	60.00		
	5	36.20	31.00	92.20	93.50
	6	4.00	2.50		
8	1	0.40	0.70		
	2	1.00	1.30		
	3	11.00	10.00		
	4	46.00	51.00		
	5	39.00	36.00	87.60	88.00
	6	2.60	1.00		

A 10 mL volume of TPB containing the virus and uranine (1mg/mL) was added to the Collison nebulizer and aerosolized into the rotating drum. After 15 minutes of cloud stabilization, a 1-min air sample (28 L) was drawn with the help of the Anderson sampler. Samples from each of the stages of the sampler were divided into two portions. The virus was quantitated by plaque assay and the concentration of the dye was measured by spectophotofluorometry.

*Stages No. 4, 5 and 6 are designed to recover particles with diameters of 2.0-3.3um, 1.0-2.0um and <1.0um, respectively.

virus in the sample would not interfere with the plaque forming ability of the other.

Poliovirus could readily grow and form countable plaques in MA-104 cells. Rotaviruses are also able to form plaques in MA-104 cells but only in the presence of trypsin in the overlay medium (Ramia and Sattar, 1979). Therefore, when the virus mixtures containing polio- and rotaviruses were to be titrated to determine the number of poliovirus PFU, an overlay medium without trypsin was used. This completely suppressed plaque formation by rotaviruses. However, for titration of the rotavirus contained in the mixtures, it was necessary to neutralize the poliovirus with the specific hyperimmune serum (1:1000) first and then perform the plaque assay with a trypsin-containing overlay.

Poliovirus could also form countable plaques in L-132 cells and in mixtures containing this virus and coronavirus, hyperimmune antiserum was used to neutralize poliovirus before the plaque assay of coronavirus. To determine the number of poliovirus PFU in such mixtures, no special pretreatment of the samples was required since poliovirus could form plaques in 48 hours at 37°C and 6 days of incubation at 33°C was necessary for the development of countable plaques by the coronavirus.

To determine whether there was any interference between the viruses, mixtures containing them were plaque assayed and the titres compared with appropriate controls. The results of these tests are presented in Table 11. There was no detectable interference between these viruses as seen by both the quantity and the quality of the plaques produced by them.

TABLE 11

PLAQUE ASSAYS TO DETECT INTERFERENCE BETWEEN VIRUSES IN THE MIXTURES USED FOR AEROSOLIZATION

Virus	PFU/mL x 10^3			
	Without Polio	Mixed with Polio	Polio Alone	Polio in Mixture
Corona 229E	3.10	3.50	0.20	0.20
Human Rota	5.00	5.40	0.90	0.87
Calf Rota (C-486)	1.20	1.30	0.16	0.16

TPB was used as the virus suspending medium. Suspensions of one virus type and virus mixtures were held at room temperature (22±1°C) for 15-20 min before plaque assay.

(2) Studies on the Airborne Survival of Rotaviruses:

Experiments with Simian Rotavirus SA-11: In the initial stages of the work with rotaviruses, SA-11 was used as a model virus and experiments were conducted to study the effect of the three different levels of RH on its survival in the airborne state. The air temperature in these experiments was maintained at 20±1°C. Figures 4-6 summarize the findings of these experiments.

In the first set of experiments in this regard the survival of the virus was tested over a period of 24 hours (Figure 4). It appeared to survive best at the medium RH level and the high RH proved to be relatively unfavorable to its infectivity in the airborne state. To further confirm and extend these preliminary observations, the experiments were repeated at least 4 times at each of the 3 RH levels and the survival of the airborne virus was examined by holding the aerosols for a 72 hour period. As is illustrated in Figure 5, the effect of various RH levels on the airborne survival of SA-11 was found to be the same as noted in the earlier experiments.

The data were further analysed in order to calculate the half-life of the virus at the various RH levels (Table 12). At the medium RH its half-life was found to be 63.52±16.19 hours. and about 45% of the infectious virus remaining detectable in the drum air even after 72 hours of aerosol age. The half-life of the SA-11 virus at the low RH level was 22.39±4.62 hours, and close to 21% of the infectious virus was recovered from the drum air 72 hours after its aerosolization. High RH was found to be the least favourable to the survival of the aerosolized virus, since 50% of the infectious virus became undetectable within 4.50±0.32 hours of aerosolization; at this RH

Figure 5. The effect of three different levels of relative humidity (20±1°C) on the airborne survival of rotavirus SA-11 over a period of 72 hours.

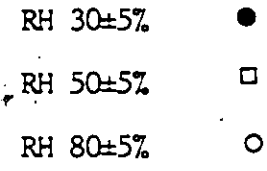
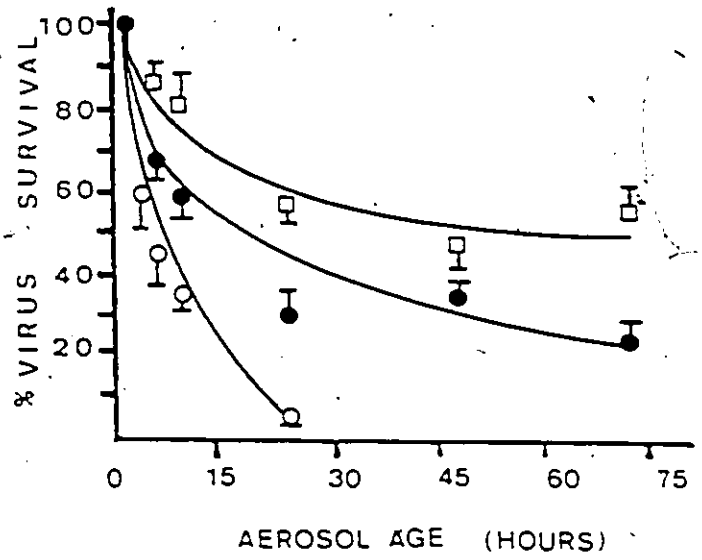


Figure 6. The effect of medium relative humidity level on the long-term airborne survival of rotavirus SA-11 at 20±1°C.


% VIRUS SURVIVAL
100
80
60
40
20
0
15
30
45
60
75
AEROSOL AGE (HOURS)

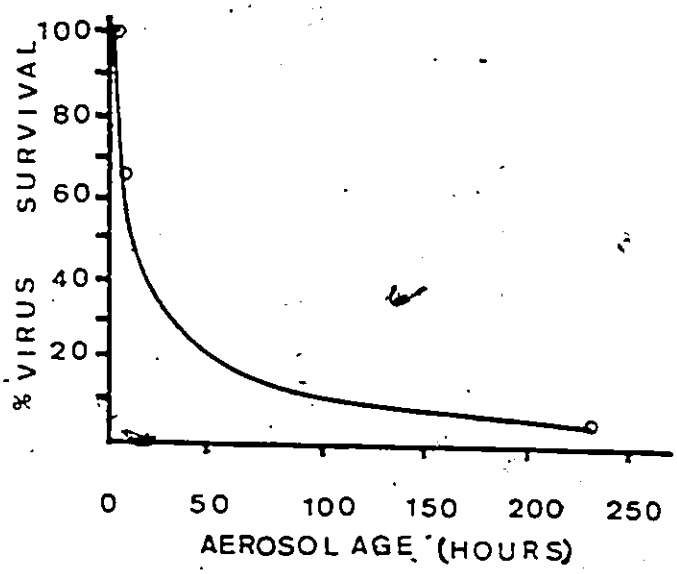

% VIRUS SURVIVAL
100
80
60
40
20
0
50
100
150
200
250
AEROSOL AGE (HOURS)

TABLE 12

HALF-LIFE IN HOURS OF AEROSOLIZED VIRUSES UNDER DIFFERENT CONDITIONS OF RELATIVE HUMIDITY AT 20 ± 1°C

Viruses	Relative Humidity		
	High (80±5%)	Mid (50±5%)	Low (30±5%)
Poliovirus type 1 (Sabin)	9.02±1.91	0	0
Rotavirus SA-11	4.50±0.32	63.5±16.2	22.4±4.62
Human Rotavirus (Wa)	9.02±1.91	27.8±10.1	18.7±3.63
Calf Rotavirus	**	24.74±3.27	18.73±3.63
Human Coronavirus 229E	3.34±0.16	67.33±8.24	26.76±6.21

** Drop in virus titre was too rapid to make possible any statistical calculations.

level, no infectious virus was recovered from the air samples collected 24 hours post aerosolization.

To determine how long infectious rotavirus SA-11 particles remained detectable in the drum air at the mid-range RH, a seperate experiment was carried out. Nearly 3% of the infectious virus could be detected in the drum air even after 223 hours (9 days) of aerosol age (Figure 6).

Comparison of the Airborne Survival of Polio-and Rotaviruses Aerosolized as a Mixture: As was mentioned earlier, a great deal of information is already available on the effect of various levels of RH on the airborne survival of polioviruses. Therefore, poliovirus type 1 (Sabin) was used as a reference to compare its airborne survival with that of the rotaviruses. In order to do this, the poliovirus and the rotavirus under test were both added to the same suspending medium and the virus mixture was aerosolized. At least three separate experiments were performed with calf (C-486) and human rotaviruses and at each one of the 3 RH levels. The results of these experiments are given in Figures 7 and 8.

When a mixture of human rotavirus and poliovirus was aerosolized and the aerosols held at 20±1°C, the half-life of the rotavirus was found to be 18.70±3.63 hours, 27.8±10.01 hours and 9.02±1.91 hours at the low, medium and high RH levels, respectively (Table 12). At the mid-range RH nearly 40% of the airborne infectious human rotavirus could be detected in the drum air at an aerosol age of 24 hours. As can seen from Figures 7 and 8 as well as Table 12, the half-life of the Canadian strain (C-486) of calf rotavirus aerosolized with poliovirus was very similar to that of human rotavirus at the low and medium RH

Figure 7. The effect of three different levels of relative humidity (20±1°C) on the airborne survival of calf rotavirus (C-486) and poliovirus type 1 (Sabin) aerosolized as a mixture.

RH 30±5% ●

RH 50±5% □

RH 80±5% ○

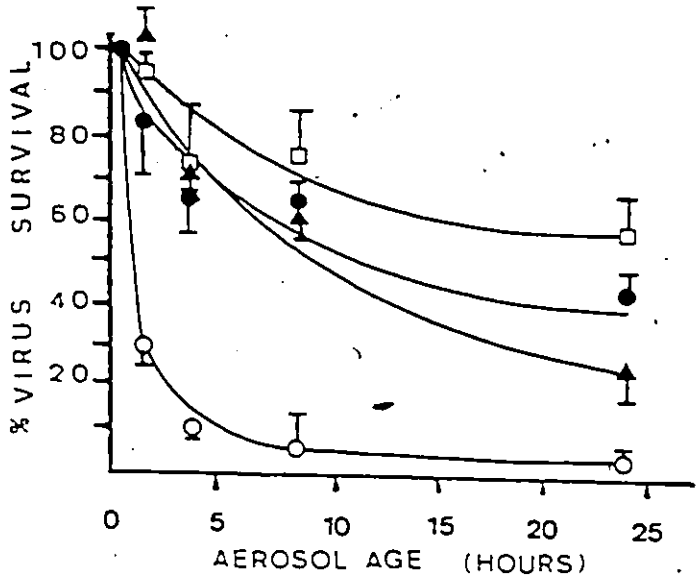
Figure 8. The effect of three different levels of relative humidity (20±1°C) on the airborne survival of human rotavirus (Wa) and poliovirus type 1 (Sabin) aerosolized as a mixture.

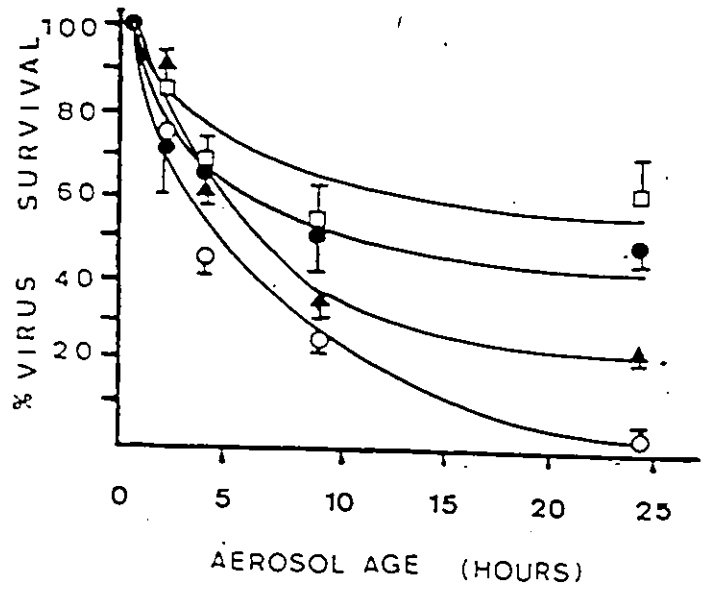
Rotavirus RH 30±5% ●

" " 50±5% □

" " 80±5% ○

Poliovirus RH 80±5% ▲


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)

levels. However, at the high RH level, the drop in the titre of the infectious calf rotavirus was too rapid and this made it difficult for us to accurately determine its half-life.

In the experiments where virus mixtures were aerosolized, the behaviour of poliovirus in the airborne state was found to be markedly different from that of rotaviruses. No infectious poliovirus could be detected in any of the samples of drum air when the aerosols were held at either the medium or low RH level. On the other hand, at the high RH, the half-life of poliovirus was found to be 9.02±1.91 hours; about 20% of the infectious virus was still present in the drum air even after 24 hours of aerosol age.

Airborne Survival of Human Rotavirus at 20±1°C: The effect of RH on the survival of human rotavirus (Wa) was tested over a period of 72 hours and the results are shown in Figure 9. At least three experiments were conducted at each of the three RH levels. It is clear that more than 40% and 20% of the virus in the cloud was still infectious at both medium and low RH respectively. Human rotavirus not only survived best at medium RH, but in comparison, its half-life was nearly 18 hours longer than that of poliovirus 1 (Sabin) at its most advantageous RH of 80±5%.

Airborne Survival of the U.K. Strain of Calf Rotavirus: Moe and Harper (1983) reported that, as compared with the low (20%) and medium (50%) RH levels, the high (90%) RH was more favorable to the airborne survival of the U.K. strain of calf rotavirus when the aerosols were held at either 10°C, 20°C or 30°C. It was, therefore, considered important to test the airborne survival of this strain of calf rotavirus in our experimental set-up so that the data generated could

be compared directly with the results of the experiments with strain C-486 of calf rotavirus.

As is evident from Figure 10, which is based on the results of a single experiment at each RH level (20±1°C), the general pattern of the effect of RH on the airborne survival of the U.K. strain was similar to that of the Canadian strain (Figure 7).

Airborne Survival of the Murine Rotavirus: The murine rotavirus was believed to be responsible for airborne outbreaks of epizootic diarrhea in infant mice kept as experimental animals (Kraft, 1957, 1958). Therefore, it was decided to include this particular type of rotavirus in this study and examine the effect of RH on its airborne survival as well. The results based on a single experiment at each one of the 3 RH levels (20±1°C) have been summarized in Figure 11. Here again, this particular rotavirus was also found to survive better at the mid-range RH and it underwent rapid inactivation when the aerosols were held at the high RH level.

Effect of Lower Air Temperature on the Airborne Survival of the Human Rotavirus: Lowering the drum air temperature to 6±1°C, further enhanced the capacity of the human rotavirus to survive in the airborne state at the medium and low RH levels (Fig.12). At this temperature, the medium RH level was also found to be most conducive for the preservation of the infectivity of airborne virus; at this RH level the half-life of the virus increased to 57.4±7.2 hours from approx. 28 hours at 20±1°C (Table 13). Even when the aerosols were held for 24 hours, there was only a 30% loss in the infectivity of the airborne virus. When compared with the results of the experiments at 20±1°C (Half-life

Figure 9. The effect of three different levels of relative humidity (20±1°C) on the the airborne survival of human rotavirus (Wa) aerosolized from TPB.

RH 30±5% ●

RH 50±5% □

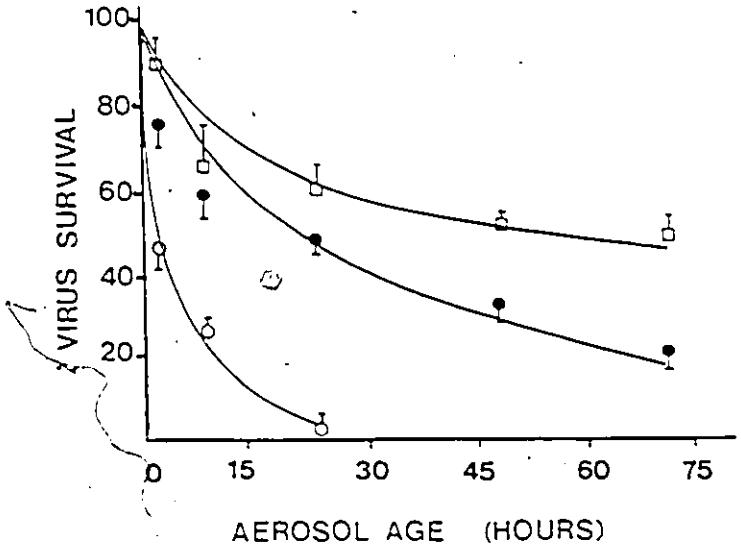
RH 80±5% ○

Figure 10. The effect of three different levels of relative humidity (20±1°C) on the airborne survival of the UK strain of calf rotavirus.

RH 30±5% ●

RH 50±5% □

RH 80±5% ○


100
80
60
40
20
% VIRUS SURVIVAL
0
15
30
45
60
75
AEROSOL AGE (HOURS)

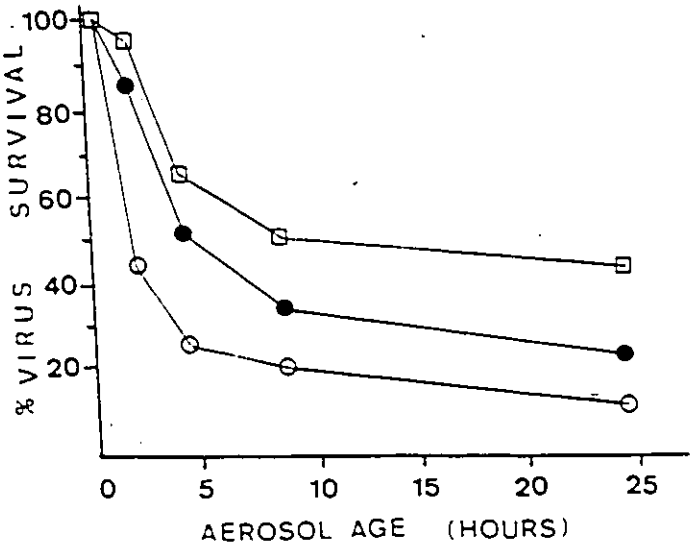

100
80
60
40
20
% VIRUS SURVIVAL
0
5
10
15
20
25
AEROSOL AGE (HOURS)

Figure 11. The effect of three different levels of relative humidity on the airborne survival of mouse rotavirus (20±1°C).

RH 30±5% ●

RH 50±5% □

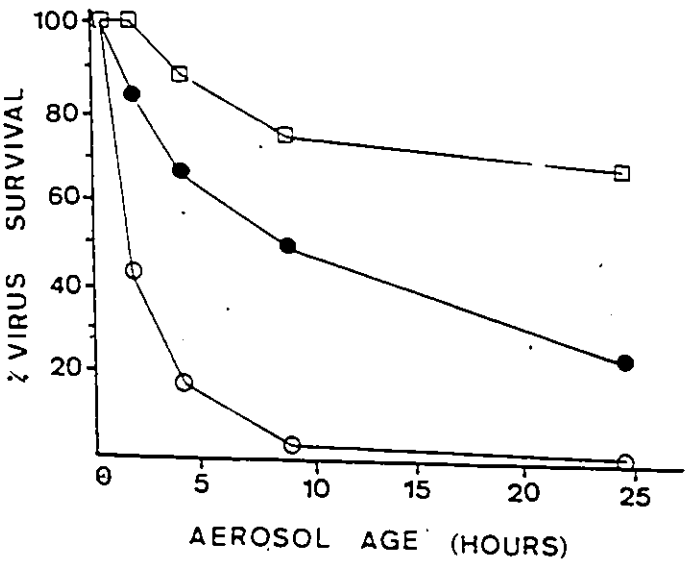
RH 80±5% ○

Figure 12. The effect of low air temperature (6±1°C) and three different levels of relative humidity on the airborne survival of human rotavirus (Wa) aerosolized from TPB.

RH 30±5% ●

RH 50±5% □

RH 80±5% ○


100
80
60
40
20
% VIRUS SURVIVAL
0
5
10
15
20
25
AEROSOL AGE (HOURS)

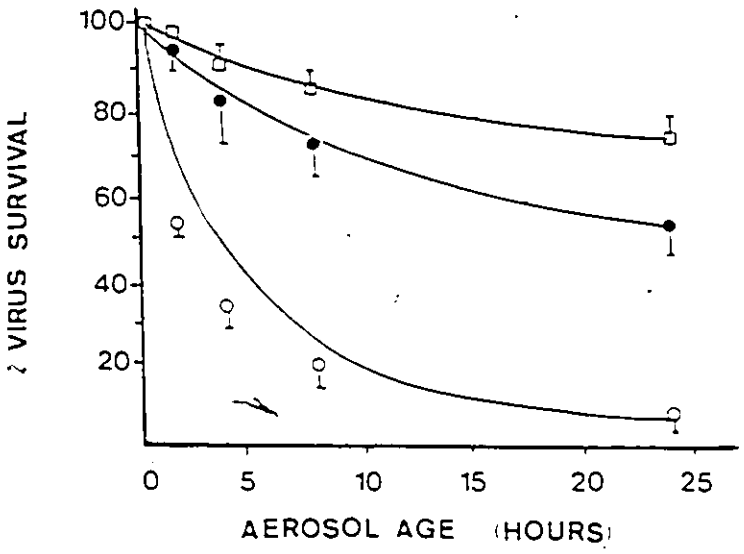

100
80
60
40
20
% VIRUS SURVIVAL
0
5
10
15
20
25
AEROSOL AGE (HOURS)

TABLE 13

HALF-LIFE IN HOURS OF AEROSOLIZED VIRUSES UNDER DIFFERENT CONDITIONS OF RELATIVE HUMIDITY AT 6 ± 1°C

Viruses	Relative Humidity		
	High (80±5%)	Mid (50±5%)	Low (30±5%)
Human coronavirus 229E	86.01±5.28	102.53±9.38	34.46±3.21
Human Rotavirus	1.71±0.70	57.40±7.20	21.6±4.00

18.7±3.63 hours), there was an increase of about 3 hours in the half-life of the virus at the low temperature when the aerosols were held at the low RH level (21.6±4.0 hours). At the lower air temperature, however, the deleterious effect of the high RH level on the airborne survival of the virus was even more pronounced (half-life = 1.71±0.7 hours).

Survival of Human Rotavirus Aerosolized from Fecal Slurry: In order to simulate the natural situations whereby rotavirus would become airborne from feces, the virus was suspended in fecal slurry and aerosolized. The aerosols were held at mid-range RH (20±1°C). The results of these experiments were then compared with the ones carried out under the same experimental conditions, but using TPB as suspending medium. As can be seen from Figure 13, the virus aerosolized from fecal slurry was found to survive better in the aerosol form when compared to that sprayed from TPB. Even at the aerosol age of 24 hours, there was < 20% loss in the infectivity of the airborne virus when it was aerosolized from fecal slurry.

3) Studies on the Airborne Survival of the Coronavirus:

Effect of Relative Humidity on the Survival of the Coronavirus at 20±1°C: The effect of RH (20±1°C) on the survival of coronavirus 229E was studied by holding the virus aerosols at the 3 different levels of RH over a period of 72 hours. At least 3 separate experiments were conducted at each of the 3 RH levels and the results of these are presented in Figure 14.

It was found that at the medium RH >50% of the virus in the air was still infectious at 72 hours when the virus aerosols were held at either the low or the medium RH. At the high RH, on the other hand, the

Figure 13. Comparison of the effect of fecal slurry and tryptose phosphate broth on the airborne survival of human rotavirus (Wa) at 50±5% relative humidity (20±1°C).

Virus aerosolized from fecal slurry ■

Virus aerosolized from TPB □

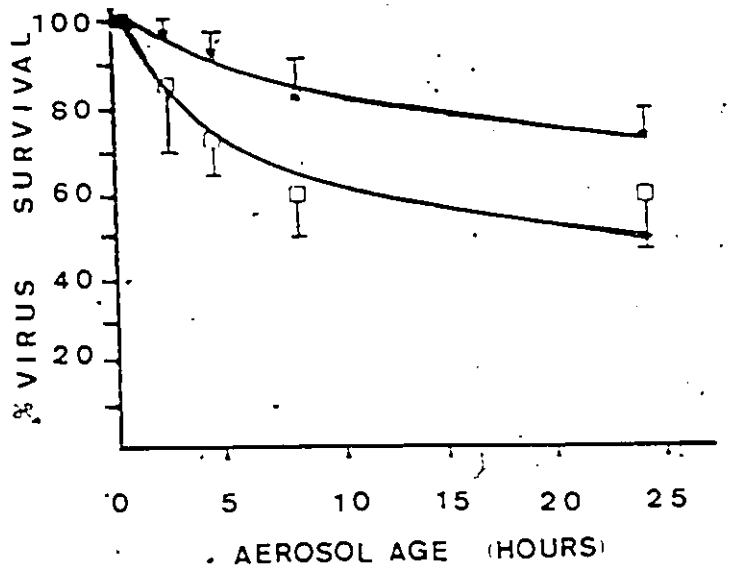
Figure 14. The effect of relative humidity (20±1°C) on the airborne survival of poliovirus type 1 (Sabin) and human coronavirus 229E aerosolized as a mixture.

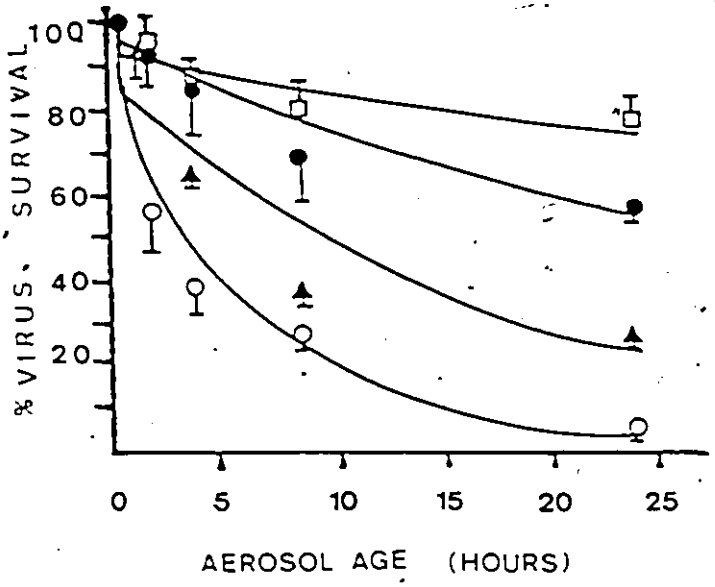
Poliovirus at RH 80±5% ▲

Coronavirus at RH 30±5% ●

" " " 50±5% □

" " " 80±5% ○


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)

half-life of the coronavirus was only 3.34±0.16 hours.

In order to determine how long the coronavirus could retain its infectivity in the drum air at 50±5% RH, two separate long term experiments were carried out. As can be seen in Figure 15, nearly 20% of the virus in the aerosol cloud was still detectable at 6 days (144 hours) of aerosol age and the half-life of the virus in these experiments was calculated to be about 70 hours.

Comparison of the Survival of the Coronavirus and the Poliovirus Aerosolized as a Mixture (20±1°C): As was done in the studies with the rotaviruses, a mixture of the coronavirus and the poliovirus was aerosolized and the aerosols held under identical conditions of RH and temperature in order to compare the effect of RH on the airborne survival of these two viruses. The results are presented in Figure 16.

In these experiments as well, no infectious poliovirus could be detected when the aerosolized mixture was held at either the low or the medium RH level. At the high RH, its half-life was again found to be about 9 hours. The pattern of the effect of the 3 different levels of RH on the airborne survival of the coronavirus was the same as described above.

Effect of Lower Air Temperature on the Airborne Survival of the Coronavirus: In 3 separate experiments at each one of the 3 RH levels, the aerosols of coronavirus were held at 6±1°C in order to study the combined effect of these 2 factors on the airborne survival of the virus. The results of these experiments are presented in Table 13 as well as Figure 17.

In comparison with the results obtained at the 20±1°C the half-life of the virus held at 6±1°Cincreased by about 30% and 53% at the

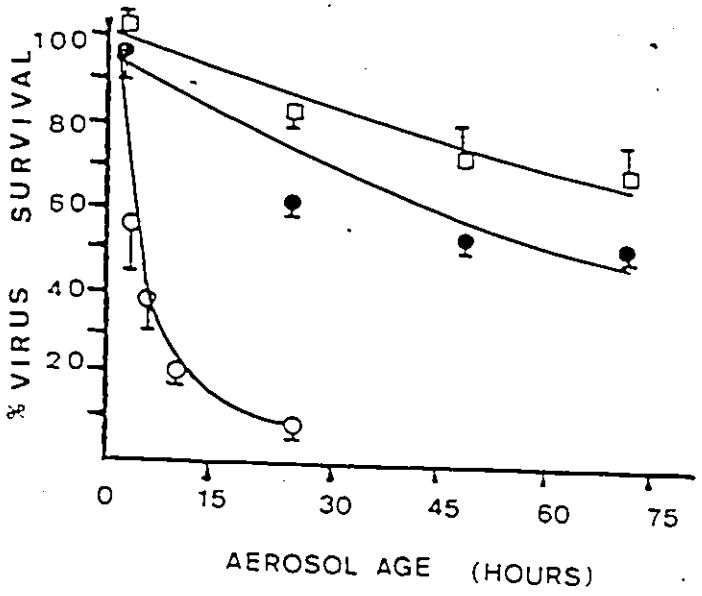
Figure 15. The effect of three different levels of relative humidity on the airborne survival (20±1°C) of coronavirus 229E over a period of 72 hours.

RH 30±5% ●

RH 50±5% □

RH 80±5% ○

Figure 16. Long-term survival (20±1°C) of airborne coronavirus 229E at 50±5% relative humidity.


% VIRUS SURVIVAL
100
80
60
40
20
0
15
30
45
60
75
AEROSOL AGE (HOURS)

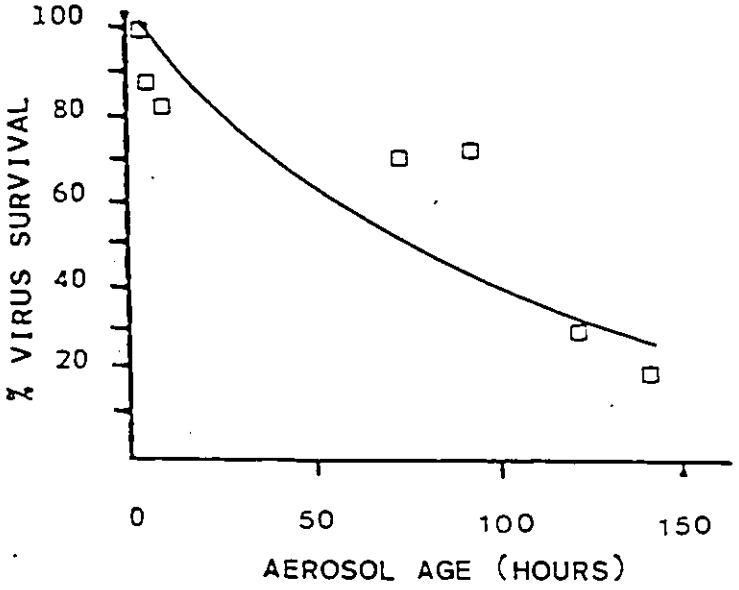

% VIRUS SURVIVAL
100
80
60
40
20
0
50
100
150
AEROSOL AGE (HOURS)

Figure 17. The effect of low air temperature (6±1°C) on the airborne survival of coronavirus 229E at three different levels of relative humidity.

RH 30±5% ●

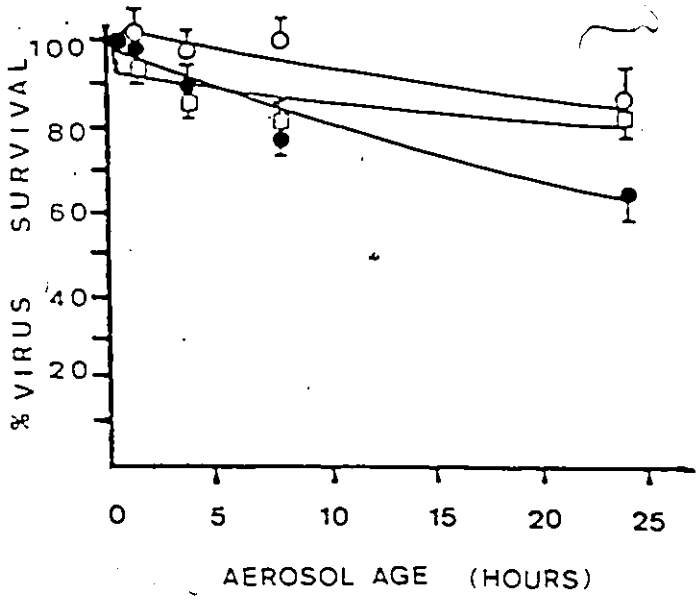
RH 50±5% □

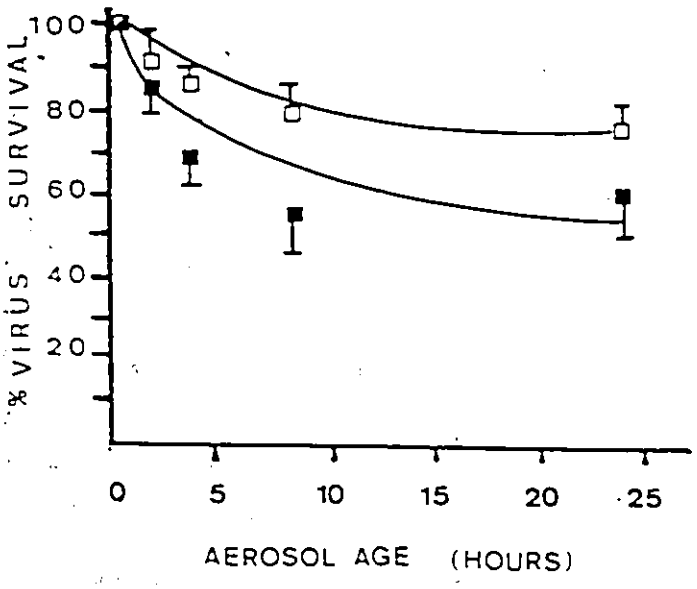
RH 80±5% ○

Figure 18. Comparison of the airborne survival of the human rotavirus and coronavirus 229E held at the medium relative humidity level (20±1°C).

Rotavirus ■

Coronavirus □


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)


% VIRUS SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
AEROSOL AGE (HOURS)

low and medium RH levels, respectively. Furthermore, in marked contrast to the very poor survival of the coronavirus at the high RH and the 20±1°C (half-life = 3.0 hours), the lowering of the air temperature to 6±1°C resulted in a dramatic increase in the capacity of this virus to survive at the high RH (half-life = 86.01±5.28 hours). Under these conditions, nearly 85% of the airborne infectious virus could be recovered at the aerosol age of 24 hours.

DISCUSSSION

It is well-established that the capacity of viruses to survive in the airborne state is modulated by a number of environmental factors. Of these, RH and air temperature have been found to be of particular significance. Although information exists (Table 1) on the influence of these factors on the airborne survival of a number of viruses of human and animal origin, when this study was initiated virtually nothing was known about the behaviour of rotaviruses and coronaviruses in the airborne state. In this section an attempt has been made to point out the reasons for the selection of the various methods and experimental conditions used in this investigation. The significance of the findings of this study has also been discussed here in relation to what is known about the epidemiology of rota- and coronavirus infections

(1) Methodology:

The high, medium and low levels of RH used in this study were chosen to represent the seasonal variations in RH levels in both indoor and outdoor environments. The experiments conducted at the medium level RH also simulated the conditions generally present in climatically controlled buildings such as modern hospitals and laboratories.

The air temperature of 20±1°C was selected to exemplify the conditions generally encountered indoors in many temperate regions. This temperature was, however, somewhat lower than what is generally regarded as room temperature (22-24°C). In our experimental set-up this was the highest air temperature that could be achieved and maintained accurately for the duration of a given experiment. This was also the limiting factor in the selection of the lower (6±1°C) air temperature.

As has been mentioned earlier, poliovirus was used as a reference virus in this study to ensure that the results generated with rota- and

coronaviruses could be compared with those of a virus whose airborne survival characteristics were better known. Here it should also be pointed out that, as far as we are aware, this is the first study where two different vertebrate viruses were aerosolized as a mixture in order to compare their airborne survival under identical experimental conditions.

Our findings on the influence of various levels of RH on the airborne survival of poliovirus are in agreement with those of earlier reports (Hemmes et al., 1960; Harper, 1961; 1963; de Jong, 1970; de Jong et al., 1973). This provides additional support to confirm that the experimental set-up in this study was not introducing any bias in the results of the airborne survival experiments on the rota- or coronaviruses.

The suitability of uranine as a physical tracer was compared directly by spraying with it rotavirus which had been labelled with ^{75}Se. This isotope was selected because of its relatively short half-life (about 120 days) so that the equipment used in these experiments would not continue to pose a radiation hazard for extended periods of time.

The particle size of virus-containing aerosols is also very important in determining their survival, physical stability, dispersion, deposition and retention in the respiratory tract (Knight, 1973). It was, therefore, essential to demonstrate that the differences seen in the survival of the airborne rota- and coronaviruses at the 3 different levels of RH were not due to variations in the size of the aerosols. The experiments using the Anderson sampler (Anderson, 1958)

clearly demonstrated that there were no major differences in the size of the aerosols being generated and aged at the 3 RH levels. The data generated in these experiments also showed that the aerosolized particles containing the dye and the virus under test belonged in the same size range.

(2) Rotaviruses:

The results of this study have revealed that the airborne survival of the 5 different types of rotaviruses tested is influenced by RH when the air temperature was maintained at 20±1°C. In this regard, the RH in the mid-range was found to be more favorable for virus survival as compared to the other 2 RH levels tested. Although experiments at the lower air temperature (6±1°C) were carried out only with the human rotavirus, the patterns of survival obtained were basically the same as seen at the 20±1°C. However, there were some important differences, namely the increased inactivation (80%) of the virus at high RH and low temperature with its half-life dropping from 9 hours (20±1°C) to 2 hours (6±1°C).

This influence of RH on airborne rotaviruses is in contrast to the earlier findings with other non-enveloped viruses such as polioviruses (de Jong et al., 1973), adenoviruses (Miller and Arstenstein, 1967), and reoviruses (Adams et al., 1982) These other viruses have been found to survive better at high RH levels. Low and medium levels of RH , on the other hand , have been found to be more conducive to the airborne survival of many types of enveloped viruses (Harper, 1961; Miller and Artenstein, 1967; Rechsteiner, 1968; Elazhary and Derbyshire, 1979a). Therefore, the behavior of rotaviruses, as seen in this study, appears to resemble that of enveloped viruses.

In nature, rotavirus aerosolization is most likely to occur from fecal matter. In order to simulate such situations, the cell culture-adapted strain of human rotavirus was suspended in feces from a laboratory-confirmed case of rotaviral diarrhea. When compared with the virus aerosolized from TPB, the presence of fecal matter in the spray fluid appeared to exert a greater protective effect on the infectivity of the airborne virus.

Here it should be noted that the fecal sample used in these experiments was first thoroughly screened for the presence of viruses which could produce cytopathology or readily visible plaques in MA-104 cells. Although the indigenous rotavirus particles present in it could be detected by electron microscopy, they were unable to form plaques. Therefore, their presence in the fecal suspension was not considered to interfere with the plaque titration of the cell culture-adapted strain of the human rotavirus added to the fecal sample.

When this study was initiated in May, 1982, there was no published information on how various environmental factors influenced the airborne survival of rotaviruses. However, about a year later, Moe and Harper (1983) published their findings on the effect of RH and temperature on the airborne survival of the UK strain of bovine rotavirus. They reported that, irrespective of the air temperature, the initial loss in the titre of the aerosolized virus was lower when the RH was either at the low (20%) or high (90%) level and that such loss was greatest under conditions of medium RH (50%). These findings, based on one single set of experiments, are in basic disagreement with our own results. The following factors may account for this discrepancy.

(a) In our study, the first air sample from the drum was collected after an aerosol stabilization period of 15 min, whereas, in their investigation air sampling began almost immediately after virus aerosolization. We considered the aerosol stabilization period essential because it allowed the large particles to settle out of air and it also permitted a more homogeneous distribution of the smaller airborne particles in the air inside the drum.

(b) To study the survival of the aerosolized rotaviruses, we sampled the air from the drum over a period of at least 24 hours. This is in contrast to a 2-hour period of aerosol aging used by the other investigators. The longer aerosol holding period used by us was more appropriate to examine the influence of RH because of the capacity of airborne rotaviruses to remain infective over extended periods of time.

(c) Initially, the lack of agreement in the results of these 2 studies was also thought to be due to basic differences in the behaviour of the Canadian and UK strains of bovine rotavirus. However, subsequent tests by us using the UK strain gave results very similar to those obtained with strain C-486. Nevertheless, it is still likely that the different cell lines used for the cultivation of this virus and basic differences in the techniques of its quantitation may have accounted for the disparity in the findings.

(d) The differences in physical tracers may also have been a contributing factor in this regard. As opposed to the bacterial spores used by Moe and Harper (1983), we employed a fluorescent dye to determine the physical loss of aerosolized particles.

A number of epidemiological observations made throughout the world indicate a correlation between the occurrence of clinical cases of

rotaviral diarrhea and the prevailing climate. In temperate zones, such cases tend to be clustered in the winter months and are uncommon or virtually absent during the summer in both animals and humans (Cheever, 1956; Heiber et at., 1978; Woode, 1982; Brandt et al., 1983; Konno et al., 1983;). Therefore, it has been suggested (Brandt et al., 1982) that a low outdoor temperature and a low indoor RH may favor the survival of rotaviruses and aid in the spread of infections caused by them. In many tropical areas, the seasonal pattern of rotaviral diarrhea is less well defined. However, in such regions also an increase in the number of cases of infections due to these viruses appears to coincide with periods of cool and dry weather (Maiya et al., 1977; deTorres et al., 1978; Black et al., 1980; Stintzing et al., 1981; Suzuki et al., 1981; Paul and Erinle, 1982).

A number of investigators have noted the involvement of the respiratory tract preceding the diarrheal phase induced by rotaviruses (Goldwater et al., 1979; Lewis et al., 1979; 1981; Nigro and Midulla, 1983). Attempts to detect these viruses in nasal swabs and throat washings have, however, been unsuccessful to date. Stals et al. (1984) reported the detection of rotavirus-specific IgA in throat swabs of children with laboratory-confirmed cases of rotaviral diarrhea. Here it should be noted that replication of rotaviruses in the respiratory tract may not be necessary before the production of enteritis; virus containing aerosols collected in the respiratory tract could be translocated by mucociliary activity and ingested (Slote, 1976). Intranasal instillation of rotavirus containing inocula has in fact been shown to result in diarrhea in piglets (Woode et al., 1976) and calves

(Light and Hodes, 1949).

Rotaviral antigens have also been detected in tracheal aspirates from cases of pneumonia (Santosham et al., 1983) and sudden infant death syndrome (Yolken and Murphy, 1983) and in middle ear aspirates from cases of otitis media (Klein et al., 1982). Gordon (1982) has suggested that rotaviruses may be able to replicate in the middle ear fluid and cause infections of the sinus, otic, mastoid and pulmonary cavities in addition to their capacity for causing diarrhea.

The observation of Kraft (1958) suggested that epizootic diarrhea of infant mice due to murine rotavirus could spread in animal colonies by virus-contaminated air. In laboratories, spread of rotaviral diarrhea in animal holding facilities is also believed to have occurred by the aerial route (Middleton et al., 1975).

The 1964 outbreak of gastroenteritis in a group of islands in the mid-pacific was shown to be due to rotavirus with the help of retrospective serological studies (Foster et al., 1980); the high attack rate and the rapid rate of spread of the outbreak strongly suggested the possiblity of aerial spread of the virus.

The role of rotavirus containing aerosols in the contamination of animate and inanimate surfaces also needs to be considered here. Depending on the RH level, the virus has been found capable of surviving on contaminated surfaces for several days (S.A. Sattar, N. Lloyd-Evans, M.K. Ijaz, R.A. Raphael, and V.S. Springthorpe. 1984. XI International Congress for Trop. Med. and Malaria, Calgary, Alberta. p. 134). Aerosolized particles of larger size generated during the handling of rotavirus-containing material (Totterdell et al., 1976) could lead to the contamination of surfaces. Such surfaces could in

turn act as a vehicle in the spread of rotaviral infections.

Rotaviruses are capable of retaining infectivity in feces for several months at room temperature (Woode, 1982) and relatively resistant inactivation by commonly used chemical disinfectants (Sattar et al., 1983). It has been found extremely difficult to prevent heavy rotaviral contamination of buildings and even of grassland (Woode, 1982). Environmental viral contamination and subsequent increased attack rate and severity of infection has been observed in a series of experiments carried out in piglets (Leece, 1978).

The process of sweeping floors has been shown to generate aerosols (Anderson et al., 1982) and re-aerosolization of virus from virus-contaminated surfaces could occur in the animal housings and hospitals (Linhares et al., 1981; Donaldson et al., 1983) by the same process or by animal or human movements. This might cause infection (1) by contamination of food and water (Woode, 1982), (2) by being inhaled and causing local respiratory tract infection, which later spread to the gastrointestinal tract by swallowing of the virus dripping down from the nasopharynx or coughed up in sputum, (3) by being inhaled and then brought up from the air passages by ciliary action and then swallowed (Flewett, 1982).

The potential threat of human infection being caused by environmental contamination with non-human rotavirus strains is unknown. A number of studies have shown experimental cross-species transmission of several types of rotaviruses including human rotavirus in animals. The possibility of zoonotic rotavirus infections of human has been suggested (Holmes, 1983; Flewett, 1984) and conversely, humans

and rodents have been proposed as a likely sourse of infection for farm animals (Woode, 1982). Although there is no documented case in the literature where animal rotavirus has been isolated from a human case, however, immunization of human subjects using live attenuated bovine rotavirus strain RIT 4237, has been reported from Finland (Vesikari et al., 1983) and Belgium (Zissis et al., 1983). In adults the vaccine did not cause clinical symptoms, and booster resulted in serum antibodies to a homologous strain. In seronegative young children one oral dose induced seroconversion to the same strain. It did not result in rotavirus excretion in stool of the vaccinated subjects. However, no protection studies have been carried out. There is no doubt that there is ample opportunity for cross species transmission of rotavirus infections.

In addition to the situations described above, generation of aerosols containing rotaviruses could also occur in a number of other settings. Rotaviruses have been detected in domestic sewage (Gerba et al., 1984), and virus-containing aerosols are known to be generated during the treatment of and spray irrigation with such wastes (Sorber, 1976; Smith and Gerba, 1980a; 1980b). Many research and service laboratories now regularly handle rotavirus-contaminated materials and several routine procedures carried out in such laboratories can lead to the production of infectious aerosols (Stern et al., 1974; Pike, 1979).

Under the experimental set-up used in our study, atmospheric conditions with medium or low levels of RH have been found to contribute greatly to the airborne survival of both human and animal rotaviruses. If naturally aerosolized rotaviruses are found to survive in the indoor atmosphere to the same extent, air could readily

facilitate their spread in settings such as hospital wards, nursing homes, day-care centres, animal houses, municipal waste treatment facilities as well as research and clinical laboratories.

At the present time no information is available on what determines the capacity of rotaviruses to survive in the airborne state when the viral aerosols are held at the low and medium RH. The higher rate of biological decay of airborne rotaviruses at 80±5% RH could be either due to the denaturation of the capsid proteins or nucleic acid (NA) components of the virus.

As mentioned in the literature review, it has been shown that infectious NA could be recovered following inactivation of the viruses in the airborne state. (de Jong, 1970; de Jong et al., 1973; 1974; Trouwborst and Kuyper, 1974). It has, therefore, been suggested that inactivation of airborne viruses occurs due to the denaturation of the protein components of non-enveloped viruses (Trouwborst et al., 1973).

The capacity of rotaviruses to survive in the airborne state could be due to a complex interplay of factors such as RH, temperature and suspending medium. Further experimentation with rotaviruses will be required to determine their inability to survive at high RH when aerosolized from TPB. Analysis of the viral RNA or capsid proteins of rotavirus particles recovered from the air sample may show the presence of gross changes in their structure. Aerosolization of the viruses from TPB dialised to remove salts is also likely to demonstrate if the deleterious effect of high RH on virus infectivity is due to the presence of salts in TPB. Similar experiments with salt solutions

may elucidate the role of TPB polypeptides in preserving rotavirus infectivity at low and medium RH.

(3) Coronavirus:

The findings reported here clearly demonstrate that HCV/229E which is generally regarded as being fragile, could readily withstand the strong shearing forces generated in the process of nebulization. This confirmed and extended the earlier observations made by Park (1980). Once aerosolized and with the aerosols maintained under certain atmospheric conditions, HCV/229E survival was even better than rotavirus survival.

At 20±1°C, the medium level of RH was found to be the most favourable for the airborne survival of this virus. In contrast to this, survival was very reduced at this temperature when the RH level was raised to 80±5%. The effect of the 3 levels of RH on the airborne survival of the coronavirus at 20±1°C was, therefore, found to be very similar to that seen with the rotaviruses (Figure 18 and Table 12). However, when the air temperature was lowered to 6±1°C, there was a dramatic change in the capacity of the coronavirus to survive at the high RH level; this was reflected by an almost 26-fold increase in its half-life. At the lower air temperature the airborne survival of this virus was further enhanced at the low and the medium RH levels.

In contrast to the results obtained with rotaviruses whereby survival is mainly associated with RH conditions, these data suggest that the airborne survival of the coronaviruses is dependent on both RH and air temperature. It might be suggested that at the lower air temperature the fluidity of the lipid-containing envelope of the virus is stabilized, thereby affording the infectious virions better

protection. Further studies will be required to investigate this and other possible mechanisms by which the airborne survival of this virus is affected. It is tempting however, to speculate on the role of environmental conditions with the seasonality of coronaviral respiratory infections. Clearly cold and damp climates would tend to favour virus transmission by the airborne route in Great Britain (Macnaughton, 1982) and in cold and dry months of the year in North America (McIntosh et al., 1970; CavalEaro and Monto, 1970).

CONCLUDING REMARKS

The study described here required approximately 3' years to complete. All but one of the initial aims of this investigation were achieved during this period. Although it was planned to include experiments on the airborne survival of field strains of human and animal rotaviruses, this could not be done because of the lack of availability of stool samples with high enough titres of infectious rotavirus particles. Samples with infectious virus titres of at least 10^6/mL are required to properly conduct the aerosol survival experiments described here. Unfortunately, the fecal samples (10% suspension) from calves and human infants which were provided to us were found to contain no more than 10^4 fluorescent focus-forming units of rotavirus/mL .

The results of the present investigation may be summarized as follows:

There has been a successful development of methodology to use in studies on the airborne survival of rota- and coronaviruses. The procedures that have been developed and standardized in the course of this work are well established and are now suitable for the study aerobiology of other viruses. The role of poliovirus 1 (Sabin) as an internal standard to compare the biological decay of rota- and coronaviruses has proven reliable. It could be employed especially in situations where mixed aerosols are being studied.

The patterns of biological decay of rotaviruses in aerosols do seem to indicate their dependence on RH conditions with a lesser dependence on temperature. The results on the airborne survival of rotaviruses have served to illustrate the potential for their aerial dissemination.

The capacity of HCV/229E to survive in the airborne state is

greatly influenced by at least two environmental parameters namely, RH and temperature. The finding that the virus can survive in air for prolonged periods under certain conditions strongly suggests the possibilty of its spread by the aerial route.

The methodology developed during the course of this study and the data obtained lay the foundations for some of the following future investigations to elucidate the role of air in the direct and indirect spread of rota- and coronaviruses:

(1) Long term experiments (>24 hours) should be carried out with the coronavirus at 6±1°C in order to explore the effect of different RH levels.

(2) The effect of body secretions and excretions on the airborne survival of coronaviruses should be attempted.

(3) In vivo transmission studies in laboratory animals by exposure to aerosolized rota- and coronaviruses should be undertaken to demonstrate the airborne transmission of these viruses and to try to establish the minimum infectious dose required for successful transmission.

(4) Studies on the airborne survival of field strains of rota- and coronaviruses should be performed to observe if they behave similarly to the laboratory-adapted strains described in the present investigation.

(5) Attempts should be made to recover these viruses from the environment using LVAS.

REFERENCES

Adams, D. J., J. C. Spendlove, R. S. Spendlove and B. B. Bennett (1982). Aerosol stability of infectious and potentially infectious reovirus particles. Appl. Environ. Microbiol. 44:903-908.

Akers, T. G. (1969). Survival of airborne virus, phage and other minute microbes. In Introduction to Experimental Aerobiology, R. L. Dimmick and A. B. Akers (Eds.), Wiley Interscience, New York, N. Y., pp. 296-339.

Akers, T. G. and M. T. Hatch (1968). Survival of a picornavirus and its infectious ribonucleic acid after aerosolization. Appl. Microbiol. 16:1811-1813.

Akers, T. G., S. Bond and L. L. Goldberg (1966a). Effect of temperature and relative humidity on survival of airborne Columbia-SK group virus. Appl. Microbiol. 14:361-364.

Akers, T. G., S. B. Bond, C. Papke and W. R. Leif (1966b). Virulence and immunogenicity in mice of airborne encephalomyocarditis virus and their infectious nucleic acids. J. Immunol. 97:379-385.

Akers, T. G., C. M. Prato and E. J. Dubovi (1973). Airborne stability of simian virus 40. Appl. Microbiol. 26:146-148.

Alford, R. H., J. A. Kasel, P. J. Gerone and V. Knight (1966). Human influenza resulting from aerosol inhalation. Proc. Soc. Exp. Biol. Med. 122:800-804.

Andries, K. M. Pensaert (1981). Vomiting and wasting disease. In Biochemistry and Biology of coronaviruses: V ter. Meulen, S. Siddell, and H. Wege (Eds.), Plenum Press, New York, pp. 399-408.

Almeida, J. D., A. E. Kulatilake and D. H. MacKay (1971). Possible airborne spread of serum-hepatitis virus within a haemodialysis unit. Lancet, ii:849-850.

Anderson, A. A. (1958). New sampler for collection, sizing and enumeration of viable airborne particles. J. Bacteriol. 76:471-484.

Anderson, R. L., D. C. Mackel, B. S. Stoler and G. F. Mallison. (1982). Carpeting in hospitals: an epidemiological evaluation. J. Clin. Microbiol. 15:408-415.

Anonymous (1970). Airborne transmission of smallpox. Brit. Med. J. 4:127.

Artenstein, M. D. and W. S. Miller (1966). Air sampling for respiratory disease agents in army recruits. Bact. Rev. 30:571-572.

Baldwin, C. L., J.F. Lemp and M. S. Barbeito (1975). Biohazards assessment in large scale zonal centrifugation. Appl. Microbiol. 29:484-490.

Barker, L. F., N. R. Shulman and R. Murray (1970). Transmission of serum hepatitis. J. Am. Med. Assoc. 211:1509-1512.

Barlow, D. F. (1972a). The aerosol stability of a strain of foot-and-mouth disease virus and the effects of stability on precipitation with ammonium sulphate, methanol or polyethylene glycol. J. Gen. Virol. 15:17-24.

Barlow, D. F. (1972b). The effects of various protecting agents on the inactivation of FMDV in aerosols and during freeze drying. J. Gen. Virol. 17:281-288.

Barlow, D. F. and A. I. Donaldson (1973). Comparison of the aerosl stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. J. Gen. Virol. 20:311-318.

Barnett, B. (1983). Viral gastroenteritis. Med. Clinics N. Am. 67:1031-1058.

Bather, R. and S. J. Webb. (1966). The role of bound water in the biological integrity of viruses and cells. In Canadian Cancer Conference, R. W. Begg., C. P. Leblond, R. L. Noble, R. J. Rossiter, R. M. Taylor and A. C. Wallace. Symp. Pergamon Press, Toronto. 6:369-380.

Bausum, H. T., S. A. Shaub, K. F. Kenyon and M. J. Small (1982). Comparison of coliphage and bacterial aerosols at a wastewater spray irrigation site. Appl. Environ. Microbiol. 43:28-38.

Baylor, E. R. and M. J. Baylor (1980). Surface-to-wind transfer of viruses. Ann. N. Y. Acad. Sci. 353:201-208.

Beard, C. W. and B. C. Easterday (1965). Aerosol transmission of hog cholera. Cornell Vet. 55:630-636.

Beard, C. W. and B. C. Easterday (1967). The influence of the route of administration of Newcastle disease virus on host response. I. Aerological and virus isolation studies. J. Inf. Dis. 117:55-61.

Bell, G. H., J. N. Davidson and H. Scarborough (1963). Textbook of Physiology and Biochemistry. E. & S. Livingston Ltd., London, England, p. 549.

Benbough, J. E. (1969). The effect of relative humidity on the survival of airborne Semliki Forest virus. J. Gen. Virol. 4:473-477.

Benbough, J. E. (1971). Some factors affecting the survival of airborne viruses. J. Gen. Virol. 10:209-220.

Benbough, J. E., and A. M. Hood (1971). Virucidal activity of open air. J. Hyg. Camb. 69:619-626.

Benda, R., M. Fuchsova and L. Danes (1962). Experimental airborne infection of monkeys with tick-borne encephalitis. Acta Virol. 6:46-52.

Berendt, R. F. and E. L. Dorsey (1971). Effect of simulated solar radiation and sodium fluorescein on the recovery of Venezuelan equine encephalitis virus from aerosols. Appl. Microbiol. 21:447-450.

Berendt, R. F., E. L. Dorsey and H. J. Hearn (1971). Virucidal properties of light and SO_2. II. Effect of a low gas concentration on aerosolized virus (36038). Proc. Soc. Exp. Biol. Med.138:1005-1008.

Berendt, R. F., E. L. Dorsey and H. J. Hearn (1972). Virucidal properties of light and SO_2. I. Effect on aerosolized Venezuelan equine encephalitis virus. Proc. Soc. Exp. Biol. Med. 139:1-5.

Berke, H. L. and T. E. Hull (1975). An improved aerosol generator. Am. Indust. Hyg. Assoc. J. 36:43-48.

Black, R.E., I. Huq, M.H. Merson, A.S.M.M. Rahman, M. Yunus, A.R.M.A. Alim, R.H. Yolken, and G.T. Curlin (1980). A two-year study of bacterial, viral, and parasitic agents associated with diarrhea in rural Bngladesh. Lancet 1:141-143.

Blanchard, D. C. and L. D. Syzdek (1982). Water-to-air transfer and enrichment of bacteria in drops from bursting bubbles. Appl. Environ. Microbiol. 43:1001-1005.

Bolton,N. E., T. A. Lincoln, J. A. Otten and W. E. Porter (1976). A method for biological testing of containment systems for viral agents. Am. Indust. Hyg. Assoc. J. 37:427-431.

von Bonsdorff, C.H., T. Hovi, A.P. Makela, L. Hovi, and M. Tevalvoto-Aarnio (1976). Rotavirus associated with acute gastroenteritis in adults. Lancet 2:423.

Brachman, P. S. (1981). Nosocomial infection control: an overview. Rev. Inf. Dis. 3:640-648.

Brain, J. D. and P. A. Valberg (1979). Deposition of aerosol in the respiratory tract. Am. Rev. Resp. Dis. 120:1325-1373.

Brandt, C.D., H.W. Kim, R.H. Yolken, A.Z. Kapikian, J.O. Arrobio, W.J. Rodriguez, R.G. Wyatt, R.M. Chanock, and R.H. Parrott (1979). Comparative epidemiology of two rotavirus serotypes and other viral agents associated with pediatric gastroenteritis. Am. J. Epidemiol. 110:243-254.

Brandt, C.D., H.W. Kim, W.J. Rodriguez, J.O. Arrobio, B.C. Jeffries, E.P. Stallings, C. Lewis, A.J. Miles, R.M. Chanock, A.Z. Kapikian, and R.H. Parrott (1983). Pediatric viral gastroenteritis during

eight years of study. J. Clin. Microbiol. 18:71-78.

Brandt, C.D., H.W. Kim, W.J. Rodriguez, J.D. Arrobio, B.C. Jeffries, and R.H. Parrott (1982). Rotavirus gastroenteritis and weather. J. Clin. Microbiol. 16:478-482.

Brock, T. D. (1974). Biology of Microorganisms, 2nd. edition, Prentice-Hall Inc., Englewood Cliffs, New Jersey, p. 453.

Brown, J., K. Cook, F. Ney and T. Hatch (1950). Influence of particle size upon the retention of particulate matter in the human lung. Am. J. Pub. Hlth. 40:450-458.

Buckland, F. E., M. L. Bynoe and D. A. J. Tyrrell (1965). Experiments on the spread of colds. II. Studies on volunteers with coxsackie virus A 21. J. Hyg. 63:327-343.

Buckland, F. E. and D. A. J. Tyrrell (1962). Loss of infectivity on drying various viruses. Nature. 195:1062-1064.

Cate, T. R., R. B. Couch, W. F. Fleet, W. R. Griffith, P. J. Gerone and V. Knight (1965). Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol. Am. J. Epidemiol. 81:95-105.

Caul, E. O., and S. K. R. Clarke (1975). Coronavirus propagated from a patients with non-bacterial gastroenteritis. Lancet 2:953-954.

Caul, E.O., and S.I. Egglestone (1977). Further studies on human enteric coronaviruses. Arch. Virol. 54:107-110.

Caul, E. O. and S. I. Egglestone (1982). Coronaviruses in humans. In Virus Infections of the Gastrointestinal Tract. D. A. J. Tyrrell and A. Z. Kapikian (Eds.), Marcel Deckker, Inc. New York, pp. 179-193.

Cavallaro, J. J. and A. S. Monto (1970). Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan. J. Infect. Dis. 122:272-279.

Centers for Disease Control (1983). Imported measles with subsequent airborne transmission in a pediatrician's office - Michigan. Morb. Mort. Wkly. Rep. 32:401-403.

Chany, C., P. Moscovici, P. Lebon, and S. Rousset (1982). Association of coronavirus infection with neonatal necrotizing enterocolitis. Pediatrics 69:209-214.

Chappel, W. A. (1960). Animal infectivity of aerosols of monkey B virus. Ann. N. Y., Acad. Sci. 85:931-934.

Cheever, F.S. (1956). Epidemic diarrheal disease of suckling mice. Ann. N.Y.Acad. Sci. 66:196-199.

Cheever, F. S. and J. H. Muller (1947). Epidemic diarrheal disease of suckling mice. I. Manifestations, epidemiology and attempts to transmit the disease. J. Exp. Med. 85:405-416.

Clark, R. P. (1983). Airborne hazards in the laboratory. Nature. 301:15-18.

Clarke, S.K.R., E.O. Caul and S.I. Egglestone (1979). The human enteric coronaviruses. Postgrad. Med. J. 55:135-142.

Collins, C. H. (1980). Laboratory acquired infections. Med. Lab. Sci. 37:291-298.

Conomy, J.P., A. Leibovitz, W. McCombs and J. Stinson (1977). Airborne rabies encephalitis: demonstration of rabies virus in the human central nervous system. Neurology 27:67-69.

Constantine, D. G. (1962). Rabies transmission by non-bite route. Pub. Hlth. Rep. 77:287-289.

Couch R. B., T. R. Cote, P. J. Gerone, W. F. Fleet, D. J. Lang, W. R.Griffith and V. Knight (1965). Production of illess with a small particle aerosol of coxsackie A 21. J. Clin. Invest. 44:535-542.

Couch, R. B., T. R. Cate, R. G. Douglas, Jr., P. J. Gerone and V. Knight (1966a). Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bact. Rev. 30:517-529.

Couch, R. B., T. C. Cate, W. F. Fleet, P. J. Gerone and V. Knight (1966b). Aerosol induced adenovirus illness resembling the naturally occurring illness in military recruits. Am. Rev. Resp. Dis. 93:529-535.

Couch, R. B., V. Knight, P. J. Gerone, T. R. Cate and R. G. Douglas (1969). Factors influencing response of volunteers to inoculation with coxsackievirus A type 21. Am. Rev. Resp. Dis. 99:24-30.

Cowen, B.S., S.B. Hitchner, and B. Lucio (1971). Characterization of a new infectious bronchitis virus isolate.1 . Serological and pathogenecity studies of Clark. Avian Dis. 15:517-519.

Cubitt, W. D. and H. Holzel (1980). An outbreak of rotavirus infection in a long-stay ward of a geriatic hospital. J. Clin. Pathol. 33:306-308.

Danes, L., J. Libich and R. Benda (1962). Experimental airborne infection of mice with tick-borne encephalitis virus. Acta Virol. 6:37-45.

Darbyshire, J. H. and D. H. Roberts (1968). Some respiratory virus and mycoplasma infections of animals. J. Clin. Path. Supplement No.

2:61-87.

Darlow, H. M. and W. R. Bale (1959). Infective hazards of water closets. Lancet i:1196-120.

Davis, W. G., A. R.Griesemer, A. J. Shadduck and L. R. Farrelle (1971). Effect of relative humidity on dynamic aerosols of adenovirus 12. Appl. Microbiol. 21:676-679.

de Jong, J. C. (1967). Phenomena of Growth and Decay with the Viruses of Poliomyelitis and Measles. Ph.D. Thesis, Utrecht, The Netherlands.

de Jong, J. C. (1970). Decay mechanisms of polio and EMC viruses in aerosols. In: Proc. Third Int. Symp. on Aerobiology. I. H. Silver (Ed.), Academic Press, New York, N. Y. p. 210.

de Jong, J. C. and K. C. Winkler (1964). Survival of measles in air. Nature (London). 201:1054-1055.

de Jong, J. C. and K. C. Winkler (1968). The inactivation of poliovirus in aerosols. J. Hyg. 66:557-565.

de Jong, J. C., T. Trouwborst and K. C. Winkler (1973). The mechanism of virus decay in aerosols. In: Airborne Transmission and Airborne Infection. J. F. Ph. Hers and K. C. Winkler (Eds.), John Wiley and Sons, New York, N.Y., pp. 124-130.

de Jong, J. C., M. Harmsen, T. Trouwborst and K. C. Winkler (1974). Inactivation of encephalomyocarditis virus in aerosols: fate of virus protein and ribonucleic acid. Appl. Microbiol. 27:59-65.

de Jong, J. C., M. Harmsen and T. Trouwborst (1975). Factors in the inactivation of encephalomyocarditis virus in aerosols. Infect. Immunity 12:29-35.

de Torres, B.V., R.M. de Ilja and J. Esparza. (1978). Epidemiological aspects of rotavirus infection in hospitalized Venezuelan children with gastroenteitis. Am. J. Trop. Med. Hyg. 27:567-572.

Donaldson, A. I. (1972). The influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. J. Gen. Virol. 15:25-33.

Donaldson, A. I. (1973). The influence of relative humidity on the aerosol stability of foot-and-mouth disease virus in aerosols from milk and fecal slurry. Res. Vet. Sci. 15:96-101.

Donaldson, A. I. (1978). Factors affecting the survival. dispersal and deposition of airborne pathogens of farm animals. Vet. Bull. 48:83-94.

Donaldson, A. I. (1979). Airborne foot-and-mouth disease. Vet. Bull.

49:653-659.

Donaldson, A. I. (1983). Quantitative data on airborne foot-and-mouth disease virus: Its production, carriage and deposition. Phil. Trans. R. Soc. Lond. B302:529-534.

Donaldson, A. I. and N. P. Ferris (1975). The survival of foot-and-mouth disease virus in open air conditions. J. Hyg. 74:409-416.

Donaldson, A. I. and N. P. Ferris (1976). The survival of some airborne animal viruses in relation to relative humidity. Vet. Microbiol. 1:413-420.

Donaldson, A. I. (1983). Experimental Aujeszky's disease in pigs: Excretion, survival and transmission of the virus. Vet. Rec. 19:490-494.

Druett, H. A. (1971). A safe method of exposing microthreads in the open air. J. Appl. Bacteriol. 34:655-658.

Druett, H.A. and K.R. May (1968). Unstable germicidal pollutant in rural air. Nature 220:395-396.

Dubovi, E. J. and T. G. Akers (1970). Airborne stability of tailless bacterial viruses S-13 and MS-2. Appl. Microbiol. 19:624-628.

Dubovi, E. J. (1971). Biological activity of the nucleic acids extracted from two aerosolized bacterial viruses. Appl. Microbiol. 21:761-762.

Edward, D. G., W. J. Elford and P. P. Laidlaw (1943a). Studies on airborne virus infections. I. Experimental technique and preliminary observations on influenza and infectious ectromelia. J. Hyg. 43:1-15.

Edward, D. G., D. Lush and R. B. Bourdillon (1943b). Studies on airborne virus infection. II. The killing of virus aerosols by ultra-violet radiation. J. Hyg. 43:11-15.

Ehrlich, R. and S. Miller (1971). Effect of relative humidity and temperature on airborne Venezuelan equine encephalitis virus. Appl. Microbiol. 22:194-199.

Ehrlich, R. and S. Miller (1972). Effect of NO_2 on airborne Venezuelan equine encephalitis virus. Appl. Microbiol. 23:481-484.

Elazhary, M. A. S. Y. and J. B. Derbyshire (1977). Effect of temperature, relative humidity and medium on the aerosol stability of infectious bovine rhinotracheitis virus. Can. J. Comp. Med. 43:158-167.

Elazhary, M. A. S. Y. and J. B. Derbyshire (1979a). Aerosol stability of bovine parainfluenza type 3 virus. Can. J. Comp. Med. 43:295-

304.

Elazhary, M. A. S. Y. and J. B. Derbyshire (1979b). Aerosol stability of bovine adenovirus type 3. Can. J. Comp. Med. 43:305-312.

Elazhary, M. A. S. Y., M. Galina, R. S. Roy, M. Fontaine and P. Lamothe (1980). Experimental infection of calves with bovine respiratory syncytial virus (Quebec strain). Can. J. Comp. Med. 44:390-395.

Estes, M. K., E. L. Palmer and J. F. Obijeski (1983). Rotaviruses: a review. Curr. Top. Microbiol. Immunol. 105:123-184.

Evans, A. S. and J. C. Niederman (1982). Epstein-Barr virus. In: Viral Infections of Humans. A. S. Evans (Ed.), Plenum Med. Book. Co., New York, N. Y., pp. 253-281.

Evans, C. G. T., R. Harris-Smith and J. E. D. Stratton (1972). The use of safety cabinets for the prevention of laboratory-aquired infection. In: Safety in Microbiology. D. A. Shapton and R. G. Board (Eds.), Academic Press, New York, N. Y., pp. 21-36.

Fairchild, G. A. (1974). Ozone effect on respiratory deposition of vesicular stomatitis virus aerosols. Am. Rev. Resp. Dis. 109:446-451.

Fannin, K. F., J. J. Gannon, K. W. Cochran and J. C. Spendlove (1977). Field studies on coliphages and coliforms as indicators of airborne animal viral contamination from wastewater treatment facilities. Water Res. 11:181-188.

Fawcett, H. H. (1972). Exposures of personnel to laboratory hazards. Am. Indust. Hyg. Assoc. J. 33:559-566.

Ferris, D.H. (1973). Epizootiology of porcine transmissible gastroenteritis (TGE). Adv. Vet. Sci. Comp. Med. 17:57-86.

Ferris, N. P. and A. I. Donaldson (1980). The influence of various chemical additives on the survival of vesicular exanthema virus in aerosols. J. Gen. Virol. 48:411-415.

Flewett, T. H. (1982). Clinical features of rotavirus infections. In Virus Infections of the Gastrointestinal Tract.D. A. J. Tyrrell and A. Z. Kapikian (Eds.), Marcel Dekker, Inc., New York, pp.125-145.

Flewett, T. H. (1984). The virology of acute infectious diarrhea. In Microbes and Infections of the Gut C.S. Goodwin (Ed.), Blackwell Scientific Publications, Boston, pp.159-185.

Flewett, T. H., A. S. Bryden, H. Davies and C. A. Morris (1975). Epidemic viral enteritis in a long-stay children's ward. Lancet. 1:4.

Flores, J., M. Sereno, A. Kalica, J. Keith, A.Z.Kapikian, and R.B.

Chanock (1984). Molecular cloning of rotaviral genes: Implications for immunoprophylaxis. In Modern Approaches to Vaccines: Molecular and Chemical Basis of Virus Virulence and Immunogenicity. R.M. Chanock and R.A. Lerner (Eds.), Cold Spring Harber Laboratory, New York, pp.159-164.

Foster, S. O., E. L. Palmer, G. W. Gary, Jr., M. L. Martin, K. L. Herrmann, P. Beasley and J. Sampson (1980). Gastroenteritis due to rotavirus in an isolated Pacific island group: an epidemic of 3,439 cases. J. Inf. Dis. 141:32-39.

Frank, G. H., R. G. Marshall and P. C. Smith (1977). Clinical and immunologic responses of cattle to infectious bovine rhinotracheitis virus after infection by viral aerosol or intramuscular inoculation. Am. J. Vet. Res. 38:1497-1502.

Frankova, V. (1975). Inhalatory infection of mice with influenza AO/PR8 virus. I. The site of primary virus replication and its spread in the respiratory tract. Acta Virol. 19:35-40.

Frankova, V. and V. Rychterova (1975). Inhalatory infections of mice with influenza AO/PR8 virus. II. Detection of virus in the blood and extrapulmonary organs. Acta. Virol. 19:35-40.

Fuchs, N. A. (1964). The Mechanics of Aerosols, revised edition. Pergamon Press, New York, N. Y.

Garwes, D.J. (1982). Coronaviruses in animals. In: Virus Infections of the Gastrointestinal Tract. D.A.J. Tyrrell and A.Z. Kapikian (Eds.). Marcel Deckker, Inc. New York. pp.315-359.

Gerba, C. P., C. Wallis and J. L. Melnick (1975). Microbiological hazards of household toilets: droplet production and the fate of residual organisms. Appl. Microbiol. 30:229-237.

Gerba, C.P., S.N. Singh and J.B. Rose (1985). Waterborne gastroenteritis and viral hepatitis. C.R.C. Critical Review. Environ. Cont. 15:213-236.

Gerne, G., G. Achilli, E. Cattaneo and P. Cereda (1978). Determination of coronavirus 229E antibody by an immune adherence haemagglutination method. J. Virol. Meth. 2:215-223.

Gerne, G., N. Passarani, P.M. Cereda, and M. Battaglia (1984). Antigenic relatedness of human enteric coronavirus OC43 :A preliminary report. J. Infect. Dis. 150:618-619.

Gerone, P.J., T.G. Ward, and W.A. Chappel (1957). Combined infections in mice with influenza virus and Diplococcus pneumoniae. Am. J. Hyg. 66:331-341.

Gerone, P. J., R. B. Couch, G. V. Keefer, R. G. Douglas, E. B. Derrenbacher and V. Knight (1966). Assessment of experimental and

natural viral aerosols. Bacteriol.Rev. 30:576-584.

Goldberg, L. J. (1971). Naval Biomedical Research Laboratory, Programmed Environment, Aerosol Facility. Appl. Microbiol. 21:244-252.

Goldberg, L. J., H. M. S. Watkins, E. E. Boerke and M. A. Chatigny (1958). The use of a rotating drum for the study of aerosols over extended periods of time. Am. J. Hyg. 68:85-93.

Goldwater, P. N., I. L. Chrystie and J. E. Banantvala (1979). Rotaviruses and the respiratory tract. Brit. Med. J. 2:1551.

Goodlow, R. J. and F. A. Leonard (1961). Viability and infectivity of microorganisms in experimental airborne infections. Bact. Rev. 25:182-187.

Gordon, A.G. (1982). Rotavirus infections and Sompe Syndrome. J. Infect. Dis. 146:117-118.

Gregory, P. H. (1973). Microbiology of the Atmosphere. 2nd. edition, Leonard Hill, London.

Greig, A.S., D. Mitchell, A.H. Corner, G.L. Bannister, E.B. Meads and R.J. Julian (1962). A hemagglutinating virus producing encephalomyelitis in baby pigs. Can J. Comp. Med. Vet. Sci. 26:49-56.

Guerin, L. F. and C. A. Mitchell (1964). A method for determining concentration of airborne virus and sizing droplet nuclei containing the agent. Can. J. Comp. Med. Vet. Sci. 28:283-287.

Gurwith, M., W. Wenman, D. Hinde, S. Feltham and H. Greenberg (1981). A prospective study of rotavirus infection in infants and young children. J. Infect. Dis. 144:218-224.

Gustafson, T. L., G. B. Lanely, E. R. Brawner, Jr., R. H. Hutcheson, Jr., P. F. Wright and W. Schaffner (1982). An outbreak of airborne nosocomial Varicella. Pediatrics 70:550-556.

Guyton, A. C. (1947). Analysis of respiratory patterns in laboratory animals. Am. J. Physiol. 150:78-83.

Gwaltney, J. M., Jr. (1980). Epidemiology of the common cold. Ann. N. Y. Acad. Sci. 353:54-60.

Hahon, N. and M. H. McGavran (1961). Airborne infectivity of the variola-vaccinia group of poxviruses for the cynomolgus monkey, Macaca irus. J. Inf. Dis. 109:294-298.

Halvorsrud, J. and I. Orstavik (1980). An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scand. J. Infect. Dis. 12:161-164.

Hara, M., J. Mukoyama, T. Tsuruhara, Y. Ashiwara, Y. Saito and I. Tagaya (1976). Acute gastroenteritis among school children associated with reovirus-like agent. Am. J. Epidemiol. 107:161-169.

Harper, G. J. (1961). Airborne microorganisms: survival tests with four viruses. J. Hyg. 59:479-486.

Harper, G. J. (1963). The influence of environment on the survival of airborne virus particles in the laboratory. Arch. ges. Virusforsch. 13:64-71.

Harper, G. J. (1965). Some observations on the influence of suspending fluids on the survival of airborne viruses. In: A Symposium on Aerobiology. R. L. Dimmick (Ed.), Naval. Biol. Lab., Oakland, Ca. pp. 335-343.

Harper, G. J. (1981). Contamination of the environment by special purpose centrifuges used in clinical laboratories. J. Clin. Pathol. 34:1114-1123.

Harris, J.R., M.L. Cohen, and E.C. Lippy (1983). Water-related disease outbreaks in the United States-1981. J. Inf. Dis. 148:759-762.

Hatch, M. T. and J. C. Warren (1969). Enhanced recovery of T-3 coliphage and Pasteurella pestis bacteriophage by means of presampling humidification technique. Appl. Microbiol. 17:685-689.

Hatch, T. F. (1961). Distribution and deposition of inhaled particles in the respiratory tract. Bact. Rev. 25:237-240.

Hausler, W. J. and R. M. Madden (1966). Microbiologic comparison of dental handpieces. 2. Aerosol decay and dispersion. J. Dental Res. 45:52-58.

Hawkes, N. (1979). Smallpox death in Britain challenges presumption of laboratory safety. Science 203:855-856.

Hearn, H. J., W. T. Soper and W. S. Miller (1965). Loss in virulence of Yellow Fever virus serially passed in HeLa cells. Proc. Soc. Exp. Biol. Med. 119:319-322.

Hearn, H. J., W. A. Chappel, P. Demchak and J. W. Daminik (1960). Attenuation of aerosolized Yellow Fever virus after passage in cell culture. Bacteriol. Rev. 30:615-623.

Heiber, J.P., S. Shelton, J.D. Nelson, J. Leon, and E. Mohs (1978). Comparison of human rotavirus disease in tropical and temperate settings. Am. J. Dis. Child. 132:853-858.

Hemmes, J. H., K. C. Winkler and S. M. Kool (1960). Virus survival as a seasonal factor in influenza and poliomyelitis. Nature (London)

188:430-431.

Henderson, D. W. (1952). An apparatus for the study of airborne infections. J. Hyg. 50:53-88.

Henderson, R. J. (1969). The outbreak of Foot-and-Mouth disease in Worcestershire. An epidemiological study: With special reference to the spread of the disease by wind carriage of the virus. J. Hyg. 67:21-33.

Henle, W., H. E. Sommer and J. Stokes (1942). Studies on airborne infection in a hospital. II. Effects of ultraviolet irradiation and propylene glycol vaporization upon the prevention of experimental airborne infection of mice by droplet nuclei. J. Pediat. 21:577-590.

Hildreth, C., M. Thomas and G. L. Ridgway (1981). Rotavirus infection in an obstetric unit. Brit. Med. J. 282:231.

Hinman, A. R., D. W. Fraser, R. G. Douglas, G. S. Bowen, A. L. Kraus, W. G. Winkler and W. W. Rhodes (1975). Outbreak of LCM virus infections in medical center personnel. Am. J. Epidemiol. 101:103-110.

Holmes, I.H. (1979). Viral gastroenteritis. Prog. Med. Virol. 25:1-36

Holmes, I.H. (1983). Rotaviruses. In The Reoviridae. W.K. Joklik (Ed.), Plenum Press, New York, pp.359-423.

Holzel, H., D. W. Cubitt, D. A. McSwiggan, P. J. Sanderson and J. Church (1980). An outbreak of rotavirus infection among adults in a cardiology ward. J. Infection. 2:33-37.

Hood, A. M. (1963). Infectvity of influenza virus aerosols. J. Hyg. 61:331-335.

Hood, A. M. (1971). An indoor system for the study of biological aerosols in open air conditions. J. Hyg. 69:607-617.

Hugh-Jones, M. E., W. H. Allen, F. A. Dark and J. G. Harper (1973). The evidence for the airborne spread of Newcastle disease. J. Hyg. 71:325-339.

Hugh-Jones, M. E. and P. B. Wright (1970). Studies on the 1967-8 Foot-and-Mouth disease epidemic: The relation of weather to the spread of disease. J. Hyg. 68:253-271.

Hyslop, N. St. G. (1965). Airborne infection with the virus of foot-and-mouth disease. J. Comp. Path. and Therap. 75:119-126.

Hyslop, N. St. G. (1970). The epizootiology and epidemiology of foot-and -mouth disease. Adv. Vet. Sci. Comp. Med. 14:261-307.

Hyslop, N. St. G. (1971). Factors influencing the epidemiol. and epizootiology of airborne dis. J. Am. Vet. Med. Assoc. 159:1500-1507.

Hyslop, N. St. G. (1973). Aerial survival and contamination of the environment by airborne filterable organisms. In: Proc. of Symp. on Viruses in the Environment and Their Potential Hazards. M. S. Mahdy and B. J. Dutka (Eds.), Canada Centre for Inland Waters, Burlington, Ont., Canada, pp. 69-85.

Hyslop, N. St. G. (1979). Observation on the survival and infectivity of airborne Rinderpest virus. Int. J. Biometerol. 23:1-7.

Ijaz, M. K., S. A. Sattar, C. M. Johnson-Lussenburg and V. S. Springthorpe (1984). Airborne survival of rotaviruses and coronaviruses: implications for disease transmission. In: Virus Diseases of Veterinary Importance in South-East Asia and the Western Pacific. A. J. Della-Porta (Ed.), Academic Press, Sydney, Aust.

Ijaz, M. K., S. A. Sattar, C. M. Johnson-Lussenburg and V. S. Springthorpe (1985). Comparison of the airborne survival of calf rotavirus & poliovirus type 1 (Sabin) aerosolized as a mixture. Appl. Environ. Microbiol. 49:289-293.

Innis, M.D. (1976). Transmission of infectious mononucleosis by blood aerosol? Lancet i:1249.

Jakab, G. J. and E. C. Dick (1967). Experimental parainfluenza (Sendai) virus infection of mice by aerosol. Bact. Proc. 76:147.

Jensen, M. M. (1964). Inactivation of airborne virus by ultraviolet irradiation. Appl. Microbiol. 12:418-420.

Jensen, M.M. (1964). Inactivation of airborne viruses by ultraviolet irradiation. Appl. Microbiol. 12:418-420.

Jericho, K. W. F., C. le Q-Darcel and E. V. Langford (1982). Respiratory disease in calves produced with aerosols of parainfluenza-3 virus and Pasteurella haemolytica. Can. J. Comp. Med. 46:293-301.

Kapikian, A.Z., R.H. Yolken, H.B. Greenberg, R.G. Wyatt, A.R. Kalica, R.M. Chanock, and H.W. Kim (1979). Gastroenteritis viruses. In, Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette and Schmidt (eds.) Am. Publ. Hlth. Assoc. pp.927-995.

Kapikian, A.Z., H.B. Greenberg, A.R. Kalica, R.G.Wyatt,.,H.W. Kim, C.D. Brandt, W.J. Rodriguez, J. Floreo, N. Singh, R.H. Parrott and R.M. Chanock (1981). New developments in viral gastroenteritis. In,Acute enteric infections in children. New prospects for the treatment and prevention. T. Holme, J. Holmgren, M.H. Merson, and R. Mollby,

(eds). Elsevier/North-Holland Biomedical, Amsterdam, pp. 9-57.

Kapikian, A.Z., R.G. Wyatt, M.M. Levine, R.H. Yolken, D. Van Kirk, R. Dolin, H.B. Greenberg and R.M. Chanock (1983). Oral administration of human rotavirus to volunteers: Induction of illness and correlates of resistance. J. Inf. Dis. 147:95-106.

Kaye, H.S., H.B. Marsh and R.W. Dowelle (1971). Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. Am. J. Epidemiol. 94:43-49.

Kaye, H.S., H.B. Marsh and W.R. Dowelle (1975). Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. Am. J. Epidemiol. 101:238-244.

Kennedy, D. A. and C. M. Johnson-Lussenburg (1975/76). Isolation and morphology of the internal component of human coronavirus, strain 229E. Intervirol. 6:197-206.

Keswick, B.H., L.K. Pickering, H.L. DuPont, and W.E. Woodward (1983). Survival and detection of rotaviruses on environmental surfaces in daycare centers. Appl. Environ. Microbiol. 46:813-816.

Klein, B.S., F.R. Dollete and R.H. Yolken (1982). The role of respiratory syncytial virus and other viral pathogens in acute otitis media. J. Pediatr. 101:16-20.

Knight, V. (1973). Airborne transmission and pulmonary deposition of respiratory viruses. In: Viral and Mycoplasma Infections of the Resp. Tract. V. Knight (Ed.), Lea & Febiger, Philadelphia, Pa., pp.1-9.

Knight, V., P. J. Gerone, W. R. Griffith, R. B. Couch, T. R. Cato, K. M. Johnson, D. J. Lang, H. E. Evans, A. Spickard and J. A. Kasel (1963). Studies in volunteers with respiratory viral agents. Am. Rev. Resp. Dis. 88:135-143.

Konno, T., H. Suzuki, N. Katsushima, A. Imai, F. Tazawa, T. Kutsuzawa, S. Kitaoka, M. Sakamoto, N. Yazaki, and N. Ishida (1983). Influence of temperature and relative humidity on human rotavirus infection in Japan. J. Infect. Dis. 147:125-128

Kraft, L. M. (1957). Studies on the etiology and transmission of epidemic diarrhea of infant mice. J. Exp. Med. 106:743-755.

Kraft, L. M. (1958). Observations on the control and natural history of the epidemic diarrhea of infant mice (EDIM). Yale J. Biol. Med. 31:121-137.

Kuehne, R. W., W. D. Sawyer and W. S. Gochenour (1962). Infection with aerosolized attenuated Venezuelan equine encephalitis virus. Am. J. Hyg. 75:347-350.

Larkin, E. P., J. T. Tierney and R. Sullivan (1976). Persistence of poliovirus 1 in soil and on vegetables irrigated with sewage wastes: Potential problems. In Virus Aspects of Municipal Waste to Land, L. B. Baldwin, J. M. Davidson and J. F. Gerber. Univ. Florida. Gainesville, Fla. 32611. pp.119-130.

Larson, E. W. (1970). Airborne transmission potential of murine leukemia infection. In: Proc. Third International Symp. on Aerobiology. I. H. Silver (Ed.), Academic Press, New York, N.Y., p. 213.

Larson, E. W., H. W. Young and J. S. Walker (1976). Aerosol evaluations of the DeVilbiss No. 40 and vaponefrin nebulizers. Appl. Environ. Microbiol. 31:150-151

Larson, E. W., J. W. Dominik and T. W. Slove (1980a). Aerosol stability and respiratory infectivity of Japanese B encephalitis virus. Inf. Immunity 30:397-401.

Larson, H.E., S.E. Reed and D.A.J. Tyrrell (1980b). Isolation of rhinoviruses and coronaviruses from 38 colds in adults. J. Med. Virol. 5:221-229.

Lawless, J. F. (1982). In Statistical Models and Methods for Lifetime Data. John Wiley Sons, New York.

Leclair, J. M., J. A. Zair, M. J. Lenin, R. G. Congdon and D. A. Goldman (1980). Airborne transmission of chickenpox in a hospital. N. Eng. J. Med. 302:450-453.

Leece, J.G., M.W. King, and W.E. Dorsey (1978). Rearing regimen producing piglet diarrhea (rotavirus) and its relevance to acute infantile diarrhea. Science 199:776-778.

Lembke, L. L., R. N. Kniseley, C. V. Nostrand and M. D. Hale (1981). Precision of the all-glass impinger and the Anderson microbial impactor for air sampling in solid-waste handling facilities. Appl. Environ. Microbiol. 42:222-225.

Lester, W. (1948). The influence of relative humidity on the infectivity of air-borne influenza A virus (PR 8 strain). J. Exp. Med. 88:361-368.

Lewis, H. M., H. B. Valman and D. A. J. Tyrrell (1979). A year's experience of the -rotavirus syndrome and its association with respiratory illness. Arch. Dis. Childhood. 54:339-346.

Lewis, H. M., H. B. Valman and D. A. J. Tyrrell (1981). Rotavirus infections and respiratory symptoms. J. Pediatrics. 98:511-512.

Lidwell, O. M., R. W. Morgan and R. E. O. Williams (1965). The epidemiology of the common cold. IV. The effect of weather. J. Hyg. 63:427-439.

Light, J.S. abd H.L. Hodes (1943). Studies on the epidemic diarrhea of the newborne: isolation of a filterable agent causing diarrhea in calves. Am. J. Pub. Hlth. 33:1451-1454.

Light, J. S. and A. L. Hodes (1949). Isolation from cases of infantile diarrhea of a filterable agent causing diarrhea in calves. J. Exp. Med. 90:113-135.

Linhares, A.C., E.P. Pinheiro, R.B. Freitas, Y.B. Gabbay, J.A. Shirley, and G.M. Beards (1981) An outbreak of rotavirus diarrhea among nonimmune isolated South American Indian community. Am. J. Epidemiol. 113:703-710.

Lippmann, M. and R. E. Albert (1969). The effect of particle size on the regional deposition of inhaled aerosols in the human respiratory tract. Am. Ind. Hyg. Assoc. J. 30:257-275.

Loosli, C. G. (1949). The pathogenesis and pathology of experimental airborne influenza virus A infections in mice. J. Inf. Dis. 84:153-168.

Loosli, C. G., H. M. Lemon, O. H. Robertson and E. Appel (1943). Experimental airborne influenza infection. I. Influence of humidity on the survival of virus in air. Proc. Soc. Exp. Biol. Med. 53:205-206.

Lycke, E., J. Blomberg, G. Berg, A. Eriksen, and L. Madsen (1978). Epidemic acute diarrhea in adults associated with infantile gastroenteritis virus. Lancet 2:1056-1057.

Macnaughton, M. R. (1982). Occurrence and frequency of coronavirus infections in humans as determined by enzyme-linked immunosorbent assay. Infect.Immun. 38:419-423.

Macnaughton, M. R. and H. A. Davies (1981). Human enteric coronaviruses. Arch. Virol. 70:301-313.

Maigetter, R. Z., R. Ehrlich, J. D. Fenters and D. E. Gardner (1976). Potentiating effects of manganese dioxide on experimental respiratory infections. Environ. Res. 11:386-391.

Maiya, P.P., S.M. Pereira, M. Mathan, P. Bhat, M.J. Albert, and S.J. Baker (1977). Aetiology of acute gastroenteritis in infancy and childhood in Southern India. Arch. Dis. Child. 52:482-485.

Marmion, B. P. and R. W. Tonkin (1972). Control of hepatitis in dialysis units. Brit. Med. Bull. 28:169-179.

Marrie, T.J., S.H.S. Lee, R.S. Faulkner, J. Ethier and C.H. Young (1982). Rotavirus infection in a geriatric population. Arch. Intern. Med. 142:313-316.

May, K. R. (1973). The Collison nebulizer: description, performance

and application. J. Aerosol. Sci. 40:235-243.

May, K. R. and H. A. Druett. (1953). The pre-impinger, a selective aerosol sampler. Br. J. Indust. Med. 10:142-151.

May, K. R. and G. J. Harper (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. Brit. J. Indust. Med. 14:287-297.

May, K. R. and H. A. Druett (1968). A microthread technique for studying the viability of microbes in a simulated airborne state. J. Gen. Virol. 51:353-366.

May, K.R., H.A. Druett and P.L. Packman (1969). Toxicity of open air to a variety of microorganisms. Nature 221:1146-1147.

Mayhew, C. J. and N. Hahon (1970). Assessment of aerosol mixtures of different viruses. Appl. Microbiol. 20:313-316.

McGarrity, G. J. and A. S. Dion (1978). Detection of airborne polyoma virus. J. Hyg. 81:9-13.

McIntosh, K. (1974). Coronaviruses: A comparative review. Curr. Top. Microbiol. Immunol. 63:85-129.

McIntosh, K., A. Z. Kapikian, H. C. Turner, J. W. Hartley, R. H. Parrott and R. M. Chanock (1970). Seroepidemiologic studies of coronavirus infection in adults and childern. Amer. J. Epidemiol. 91:585-592.

McKissick, G. E., R. A. Griesemer and R. L. Farrell (1970a). Aerosol transmission of Rauscher murine leukemia virus. J. Natl. Cancer Inst. 45:625-636.

McKissick, G. E., L. G. Wolf, R. L. Farrell, R. A. Griesemer and A. Hellman (1970b). Aerosol transmission of oncogenic viruses. In: Proc. Third International Symp. on Aerobiology. I. H. Silver (Ed.), Academic Press, New York, N.Y., p. 233.

McNulty, M. S.(1978). Rotaviruses. J. Gen. Virol. 40:1-18.

McVicar, J. W. and R. J. Eisner (1983). Aerosol exposure of cattle to foot-and-mouth disease virus. J. Hyg. 91:319-328.

Med. Res. Coun. Canada (1980). Containment requirement for experiments involving recombinant DNA or animal viruses or cells. In Guideline for the Recombinant DNA molecule and Animal viruses and Cells. Minister of Supply and Services, Canada. pp. 21-35.

Merekalova, Z. I., N.M. Orekhova and E. I. Zharona (1981). Possible ways of excreting murine leukemia virus into the environment and the so-called input gate for infection. Eksp. Onkol. 3:59-62.

Middleton, P. J., M. Petric and M. T. Szymanski (1975). Propagation of infantile gastroenteritis virus (orbi-group) in conventional and germ-free piglets. Inf. Immunity 12:1267-1280.

Miller, W. S. (1966). Infection of pigeons by airborne Venezuelan equine encephalitis virus. Bact. Rev. 30:589-596.

Miller, W. S. and M. S. Artenstein (1967). Aerosol stability of 3 acute respiratory disease viruses. Proc. Soc. Exp. Biol. Med.125:222-227.

Miller, W. S., P. Demchak, C. R. Rosenberger, J. W. Dominik and J. L. Bradshaw (1963). Stability and infectivity of airborne yellow fever and rift valley fever viruses. Am. J. Hyg. 77:114-121.

Mitchell, C. A. and L. F. Guerin (1972). Influenza A of human, swine equine and avian origin: comparison of survival in aerosol form. Can. J. Comp. Med. 36:8-11.

Mitchell, C. A., L. F.Guerin and J.Robillard (1968). Decay of influenza A viruses of human and avian origin. Can. J. Comp. Med. 32:544-546.

Moe, K. and J.A. Shirley (1982). The effects of relative humidity and temperature on the survival of human rotavirus in faeces. Arch. Virol.72:179-186.

Moe, K., & G. J. Harper (1983). Effect of RH and temperature on the survival of bovine rotavirus in aerosol. Arch. Virol. 76: 211-216.

Monto, A.S. (1982). Coronaviruses. In Viral Infections of Humans: Epidemiology and Control. A.S. Evans (Ed.), Plenum Med. Book Co., New York, pp.151-165.

Moore, B., P. Lee, M. Hewish, B. Dixon, and T. Mukherjee (1977). Coronaviruses in training centre for intellectually retarded. Lancet i: 261-263.

Moore, B. E., B. P. Sagik and C. A. Sorber (1979). Procedures for the recovery of airborne human enteric viruses during spray irrigation of treated wastewater. Appl. Environ. Microbiol. 38:688-693.

Morrow, P. E. (1980). Physics of airborne particles and their deposition in the lung. Ann. N. Y. Acad. Sci. 353:71-80.

Moscovici, O., C. Chany, P. Lebon, S. Rousset, and Laporte (1980). Association d'infection a coronavirus avec l'entercolite hemorragique du nouveau-ne. C.R. Acad. Sc.[D] (Paris) 290:869-872.

Moser, M. R., T. R. Bender, H. S. Margolis, G. R. Noble, A. P. Kendal and D. G. Ritter (1979). An outbreak of influenza aboard a commercial airliner. Am. J. Epidemiol. 110:1-6.

Murphy, A., M. Albrey, and E. Crewe. (1977). Rotavirus infection in neonates. Lancet ii: 1149-1150.

Nigro, G. and M. Midulla (1983). Acute laryngitis associated with rotavirus gastroenteritis. J. Inf. 7:81-82.

Nir, Y. D. (1959). Airborne West Nile virus infection. Am. J. Trop. Med. Hyg. 8:537-539.

Nir, Y., A. Beemer and R. A. Goldwasser (1965). West Nile virus infecton in mice following exposure to a viral aerosol. Brit. J. Exp. Pathol. 46:443-449.

Omerod, E., I. A. McCandlish and O. Jarrett (1979). Disease produced by feline caliciviruses. Vet. Rec. 104:65-69.

Paul, M.O. and E.A. Erinle (1982). Influence of humidity on rotavirus prevalence among Nigerian infants and young children with gastroenteritis. J. Clin. Microbiol. 15:212-215.

Park, G. W. (1980). The use of a large volume air sampler for the detection of airborne respiratory viruses: a feasibility study. M. Sc. Thesis, Univ. Ottawa, Ottawa, Canada.

Parker, J.C., S.S. Crosse and W.P. Rowe (1970). Rat coronavirus (RCV): A prevalent, naturally occurring pneumotropic virus of rats. Arch. Ges. Virusforsch. 31:293-302.

Pensaert, M.B. and P. de Bouck (1978). A new coronavirus-like particle associated with diarrhea in swine. Arch. Virol. 58:243-247.

Perkins, J. E., A. M. Bahlke and H. F. Silverman (1947). Effect of ultraviolet irradiation of classrooms on spread of measles in a large rural central school. Am. J. Pub. Hlth. 37:529-537.

Peto, S. and E. Powell (1970). The assessment of aerosol concentration by means of the Anderson sampler. J. Appl. Bact. 33:582-598.

Phillips, G. B. (1965a). Safety in the chemical laboratory. XIII. Microbiological hazards in the laboratory. Part one - Control. J. Chem. Education 42:A43-A48.

Phillips, G. B. (1965b). Safety in the chemical laboratory. XIII. Microbiological hazards in the laboratory. Part two - Prevention. J. Chem. Education 42:117-130.

Phillip, J.I.H., S.F. Cartwright and A.C. Scott (1971). The size and morphology of transmissible gastroenteritis and vomiting and wasting disease of pigs. Vet. Rec. 88:311-312.

Pike, R. M. (1979). Laboratory-associated infections: incidence, fatalities, causes and prevention. Ann. Rev. Microbiol. 33:41-66.

Rabey, F., R.J. Janssen and L.M. Kelley (1969). Stability of St. Louis encephalitis virus in the airborne state. Appl. Microbiol. 18:880-882.

Ramia, S. and S. A. Sattar (1979). Simian rotavirus SA-11 plaque formation in the presence of trypsin. J. Clin. Microbiol. 10:609-614.

Ramia, S. and S. A. Sattar (1980). Concentration of seeded Simian rotavirus SA-11 from potable waters by using talc-celite layers and hydroextraction. Appl. Environ. Microbiol. 39:493-499.

Raphael, R.A., S.A. Sattar and V.S. Springthorpe (1983). Rotavirus inactivation by chemical disinfectants and antiseptics used in microbiological laboratories. Proc. Ann. Assoc. Biol. Safety Conf. Ottawa, Ont. 170-183.

Raphael, R.A., S.A. Sattar and V.S. Springthorpe (1985). Long- term survival of human rotavirus in raw and treated river water. Can.J. Microbiol. 31:124-128.

Ratanasethakul, C. and R. B. Cumming (1983). The effect of route of infection and strain of virus on the pathology of Australian infectious bronchitis. Aust. Vet. J. 60:209-213.

Rechsteiner, J. (1968). Phenomena of decay in respiratory syncytial virus. Ph.D. thesis, Utrecht, The Netherlands.

Reed, S.E. (1984). The behaviour of recent isolates of human respiratory coronavirus in vitro and in volunteers: Evidence of heterogeneity among 229E-related strains. J. Med. Virol. 13:179-192.

Rettig, P.J. and G.P. Altshuler (1985). Fatal gastroenteritis associated with coronavirus-like particles. Am. J. Dis. Child. 139:245-248.

Riley, E. C., G. Murphy and R. L. Riley (1978). Airborne spread of measles in a suburban elementary school. Am. J. Epidemiol. 107:421-432.

Riley, R. L. (1974). Airborne infections. Am. J. Med. 57:466-475.

Riley, R. L. (1977). Ultraviolet air disinfection for protection against influenza. The Johns Hopkins Med. J. 140:25-27.

Riski, H. and T. Hovi (1980). Coronavirus infections of man associated with diseases other than the common cold. J. Med. Virol. 6:259-265.

Robb, J. A. and C. W. Bond (1979). Coronaviridae. In Comprehensive Virology. J. Fraenkel-Conrat and R. R. Wagner (Eds.), Plenum Press, New York, 14:193-247.

Robinson, E., J. A. MacLeod and C. E. Lapple (1959). A meterological tracer technique using uranine dye. J. Meterol. 16:63-67.

Rousset, S, O. Moscovici, P. Lebon, J.P. Barbel, P. Helardao, B. Mace, F.Bargy, Le Tan Vinh, and C. Chany (1984). Intestinal lesions containing coronavirus-like particles in neonatal necrotizing enterocolitis: An ultrastructural analysis. Pediatrics 73:218-224.

Rutter, D. A. and C. G. T. Evans (1972). Aerosol hazards from some clinical laboratory apparatus. Brit. Med. J. 1:594-597.

Ryder,R.W., J.E. McGowan, M.H..Hatch, and E.L. Palmer (1977). Reovirus-like agent as a cause of nosocomial diarrhea in infants. J. Pediatr. 90:698-702.

Santosham, M., R.H. Yolken, E. Quiroz, L. Dillman, G. Oro, W.C. Reeves and R.B. Sack (1983). Detection of rotavirus in respiratory secretions of children with pneumonia. Clin. Lab. Obs. 103:583-585.

Sattar, S.A., R.A. Raphael and V.S. Springthorpe (1983). Rotaviruses and chemical disinfectants. In Proc. Fourth International Symposium on Neonatal Diarrhea, Saskatoon, Sask., S.D. Acres, A.J. Forman and H.H. Fast (eds.) p. 90-99

Sattar, S. A., M. K. Ijaz, C. M. Johnson-Lussenburg and V. S. Springthorpe (1984a). Effect of relative humidity on the airborne survival of rotavirus SA-11. Appl. Environ. Microbiol. 47:879-881.

Sattar, S.A., R.A. Raphael and V.S. Springthorpe (1984b). Rotavirus survival in conventionally-treated drinking water. Can. J. Microbiol. 30:653-656.

Schaffer, F. L., M. E. Soergel and D. C. Straube (1976). Survival of airborne influenza virus: effects of propagating host, relative humidity, and composition of spray fluids. Arch.Virol. 51:263-273.

Schechmeister, I. L. (1950). Studies on the experimental epidemiology of respiratory infections. J. Infect. Dis. 87:128-132.

Schnagl, R.D., I.H. Holmes and M. Scollay (1978). Coronavirus-like particles in aboriginals and non-aboriginals in Western Australia. Med.J. Aust. 1:307-310.

Schulman, S. L. and E. D. Kilborne (1963). Experimental transmission of influenza virus infection in mice. I. The period of transmissibility. J. Exp. Med. 118:257-275.

Scott, G.H. and R.J. Sydiskis (1976). Responses of mice immunized with influenza virus by aerosol and parenteral routes. Infect. Immunity 13:696-703

Sevoian, M., D. M. Chamberlain and R. H. Yarose (1963). Avian lymphomatosis. V. Airborne transmission. Avian Dis. 7:102-105.

Shaffer, J. G. (1964). III Airborne infection in hospitals. Am. J. Pub. Hlth. 54:1674-1682.

Shipe, E. L., M. E. Tyler and D. N. Chapman (1959). Bacterial aerosol samplers. II. Development and evaluation of the Shipe sampler. Appl. Microbiol. 7:349-354.

Shrene, W. B. and H. D.Tow, Jr. (1981). Bacteriological and serological surveillance of dentists exposed to dental aerosols. Bull. Tokyo Dent. Coll. 22:151-157.

Siddell, S. (1983). The biology of Coronaviruses. J. Gen Virol. 64:761-776.

Siddell, S., H. Wege, and V. ter Meulen (1982). The structure and replicaton of coronaviruses. Curr. Top. Microbiol. Immunol. 99:131-164.

Slobodenyuk, V. K. and G. I. Karukhin (1970). Experimental substantiation of aerosol method of disinfection during viral infections. II. Inactivating action of the aerosols of hydrogen peroxide, chloramine and hexylresorcine on different viruses in the air and on surfaces. Zh. Mikrobiol. Epidemiol. Immunobol. 47:113-117.

Slote, L. (1976). Viral aerosols: a potential occupationally related health threat in aerated wastewater treatment systems. J. Environ. Hlth. 38:310-314.

Smith, E. M., and C. P. Gerba (1980a). Detection of rotavirus in raw and treated wastewater. In Proc.179th National Meeting Am.Chem.Soc. 20:167-169.

Smith, E. M., and C. P. Gerba (1980b). Survival and detection of rotaviruses in the environment. In Proc.3rd International Symp. Neonatal fDiarrhea. S. D. Acres, A. J. Forman, H. Fast (Eds.), VIDO Univ.Sask., Saskatoon, Sask. pp. 67-81.

Smith, E.M. and C.P. Gerba (1982). Development of a method for detection of human rotavirus in water and sewage. Appl. Environ. Microbiol. 43:1440-1450.

Snodgrass, D.R. and J.A. Herring (1977). The action of disinfectants on lamb rotavirus. Vet. Record 101:81.

Songer, J. R. (1967). Influence of relative humidity on the survival of some airborne viruses. Appl. Microbiol. 15:35-42.

Sorber, C.A. (1976). Viruses in aerosolized waste water. In:Virus Aspects of Applying Municipal Waste to Land. L.B. Baldwin, J.M.

Davidson and J.F. Gerber (Eds.), Univ. Florida, Gainsville, Fla. 32611 pp. 83-86.

Spendlove, J. C. and K. F. Fannin (1982). Methods of characterization of virus aerosols. In: Methods in Environmental Virology. C. P. Gerba and S. M. Goyal (Eds.), Marcel Dekker Inc., New York, N.Y., pp. 261-329.

Stals, F., F.J. Walther and C.A. Bruggeman (1984). Faecal and pharyngeal shedding of rotavirus and rotavirus IgA in children with diarrhea. J. Med. Virol. 14:333-339.

Stern, E. L., J. W.Johnson, D. Vesley, M. M. Halbert, L. E. Williams and P. Blume (1974). Aerosol production associated with clinical laboratory procedures. Am. J. Clin. Path. 62:591-600.

Stephenson, E.H., E.W. Larson and J.W. Dominik (1984). Effect of environmental factors on aerosol-induced Lassa virus infection. J Med. Virol. 14:295-303.

Stinzing, G., E. Back, B. Tufvesson, T. Johnson, T Wadstrom, and D. Habte. (1981). Seasonal fluctuations in the occurrence of enterotoxigenic bacteria and rotavirus in paediatric diarrhoea in Addis Ababa. Bull. W.H.O. 59:67-73.

Strange, R.E., J.E. Benbough, P. Hambleton, and K.L. Martin (1972). Methods for the assessment of microbial populations recovered from enclosed aerosols. J. Gen. Microbiol. 72:117-125.

Stuart, B. O. (1973). Deposition of inhaled aerosols. Arch. Internal Med. 131:60-73.

Sturman, L.S. and K.V. Holmes (1983). The molecular biology of coronaviruses. Adv. Virus Res. 28:35-112.

Sulkin, S. E. (1961). Laboratory-acquired infections. Bact. Rev. 25:203-209.

Sulkin, S. E. and R. M. Pike (1949). Viral infections contracted in the laboratory. New Eng. J. Med. 241:205-213.

Sutmoller, F., R.S. Azeredo, M.D. Lacerda, O.M. Barth, H.G. Pereira, E. Hoffer, and H.G. Schatzmayr (1982). An outbreak of gastroenteritis caused by both rotavirus and Shigella sonnei in a private school in Rio de Janeiro. J. Hyg.88:285-293.

Suzuki, H., Y. Amano, H. Kinebuchi, E.G. Vera, A. Danila, J. Lopez, R. Gustabo, T. Komno, and N. Ishida (1981). Rotavirus infection in children with acute gastroenteritis in Ecuador. Am. J.Trop. Med. Hyg. 30:293-294.

Tan, J.A. and R.D. Schnagl (1981). Inactivation of a rotavirus by disinfectants. Med. J. Australia 1:19-23.

Tajima, M. (1970). Morphology of transmissible gastroenteritis virus of pigs. Arch. Ges. Virusforsch. 29:105-108.

Teltsch, B. and E.Katznelson (1978). Airborne enteric bacteria and viruses from spray irrigation with wastewater. Appl. Environ. Microbiol. 35:290-296.

Teshima, T., T. Isawa, T. Hirano, A. Ebina and K. Komno (1981). Measurement of aerosol size and its effect on inhaled deposition patterns in the lungs. Japan J. Nucl. Med. 18:449-454.

Thomas, G. (1970a). An adhesive surface sampling technique for airborne viruses. J. Hyg. 68:273-282.

Thomas, G. (1970b). Sampling of rabbitpox aerosols of natural origin. J. Hyg. 68:511-517.

Thomas, G. (1970c). Sampling of airborne viruses. In: Proc. Third International Symp. on Aerobiology. J. H. Silver (Ed.), Academic Press, New York, N.Y., p.266-276.

Tilloison, J. R., F. Axelord and D. O. Lyman (1977). Morbid. Mortal. Wkly. Rep. 26:249.

Tomlinson, A. J. H. (1957). Infected airborne particles liberated on opening screw-capped bottles. Brit. Med. J. II:15-17.

Totterdell, B.M., I.L. Christie and J.E. Banatvala. (1976). Rotavirus infections in a maternity unit. Arch. Dis. Child. 51:924-928.

Trouwborst, T., and J.C. de Jong (1973a). Interaction of some factors in the mechanism of inactivation of bacteriophage MS2 in aerosols. Appl. Microbiol. 26:252-257.

Trouwborst, T., J. C. de Jong and K. C. Winkler (1973b). Inactivation of the enzyme trypsin in aerosols. J. Colloid Interface Sci. 45:198-208

Trouwborst, T. and S. Kuyper (1974). Inactivation of bacteriophage T-3 in aerosols: effect of prehumidification on survival after spraying from Trouwborst, T., S. Kuyper, J. C. de Jong and A. D. Plantinga (1974). Inactivation of some bacterial and animal viruses by exposure to liquid air interfaces. J. Gen. Virol. 24:155-165.

Tyler, M. E. and E. L. Shipe (1959). Bacterial aerosol samplers. I. Development and evaluation of the All Glass Impinger. Appl.Microbiol. 7:337-349.

Tyrrell, D.A.J., J.D. Almeida, D.M. Berry, C.H. Cunningham, D. Hamre, M.S. Hofstad, L. Mallucci and K. McIntosh (1968). Coronaviruses. Nature (Lond.). 220:650.

Turner, A. G., J. R. Wilkins III and J. G. Craddock (1975). Bacterial aerosolization from an ultrasonic cleaner. J. Clin. Microbiol. 1:289-293.

Udenfriend, S. (1964). Fluoresence Assay in Biology and Medicine. Vol.11. Academic Press, New York.

Urasawa, S., T. Urasawa, and K. Taniguchi (1981) Sequential passages of human rotavirus in MA-104 cells. Microbiol. Immunol. 25:1025-1035

Vesikari, T., E. Isolauri, A. Delem, E. D'Hondt, F.E. Andre and G. Zississ (1983). Immunogenicity and safety of live oral attenuated bovine rotavirus vaccine strain RIT 4237 in adults and young children. Lancet 11: 807-811.

Walker, J.S., E.L. Stephen and R.O.Spertzel (1976). Small particle aerosols of antiviral compounds in treatment of type A influenza pneumonia in mice. J. Infect. Dis. 133 (Suppl.): A140-A144.

Ward, B. K., C. M. Chenoweth and L. G. Irving (1982). Recovery of viruses from vegetable surfaces. Appl. Environ. Microbiol. 44:1389-1394.

Wardley, R. C. and R. C.Poney (1977). Aerosol transmission of feline caliciviruses: an assessment of its epidemiological importance. Brit. Vet. J. 133:504-508.

Warren J. C., T. G. Akers and E. J. Dubovi (1969). The effect of prehumidification on sampling of selected airborne viruses. Behaviour of Colorado tick fever, vesicular stomatitis, neurovaccinia and encephalomyocarditis viruses in the airborne state. In: Symposium on Aerobiology. R. L. Dimmick (Ed.), Nav. Biol. Lab., Nav. Supply Centre, Oakland, Ca., pp. 381-388.

Watkins, H.M.S., L.J. Goldberg, E. F. Deig and W.R.Leif (1965). Behaviour of Colorado Tick Fever, Vesicular Stomatitis, Neurovaccinia and Encephalomyocarditis viruses in the airborne state. Proc. First Intern. Symp. Aerobiol., Berkley, Calif. pp.381-388.

Webb, S. J. (1959). Factors affecting the viability of air-borne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. 5:649-669.

Webb, S. J. (1963). Possible role of water and inositol in the stucture of nucleoproteins. Nature 198:785.

Webb, S. J., R. Bather and R. W. Hodges (1963). The effect of relative humidity and inositol on airborne viruses. Can. J. Microbiol. 9:87-92.

Wedum, J. G. (1984). Airborne infection in the laboratory. Am. J.

Pub. Hlth. 54:1669-1673.

Wege, H., St. G. Siddell and V. terMeulen (1982). The biology and pathogenesis of coronaviruses. Curr. Topic. Microbiol. Immunol. 99:165-200.

Wehrle, P. F., J. Posch, K. H. Richter and D. A. Henderson (1970). An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. Bull. World Hlth. Org. 43:669-679.

Wells, W. F. and W. R. Stone (1934). An airborne infection study. II. Droplets and droplet nuclei. Am. J. Hyg. 20:611-627.

Wells, W. F. and H. W. Brown (1936). Recovery of influenza virus suspended in air and its destruction by ultraviolet radiation. Am. J. Hyg. 24:407-413.

Wells, W. F. and W. Henle (1941). Experimental airborne disease. Quantitative inoculation by inhalation of influenza virus. Am. J. Hyg.48:298-301.

Wells, W. F., M. W. Wells and T. S. Wilder (1942). The environmental control of epidemic contagion. I. An epidemiologic study of radiant disinfection of air in day schools. Am. J. Hyg. 35:97-121.

Westwood, J. C. N. (1963). Smallpox in Bradford. Proc. Roy. Soc. Med. 56:346.

Westwood, J. C. N., E. A. Boulter, E. T. W. Bowen and H. B. Maber (1966). Experimental respiratory infection with poxviruses. Brit. J. Exp. Path. 47:453-465.

White, L. A., D. J. Hadley, D. E. Davids and R. Naylor (1975). Improved large volume sampler for the collection of bacterial cells from aerosol. Appl. Microbiol. 29: 335-339.

White, R. J. and S. H. Madin (1964). Pathogenesis of murine hepatitis: route of infection and susceptibility of the host. Am. J. Vet. Res. 25:1236-1240.

Whitewell, F., P. J. Taylor and A. J. Oliver (1957). Hazards to laboratory staff in centrifuging screwcapped containers. J. Clin. Path. 10:88-91.

Wilkinson, P. J. and A. I. Donaldson (1977). Transmission studies with African swine fever virus. The early distribution of virus in pigs infected by airborne virus. J. Comp. Path. 87:497-501.

Wilkinson, P. J. and A. I. Donaldson, A. Greig and W. Bruce (1977). Transmission studies with African swine fever virus. Infection of pigs by airborne virus. J. Comp. Path. 87:487-495.

Winkler, K. C. (1973). The scope of aerobiology. In Airborne Transmission and Airborne Infection, J. F. Ph. Hers and K. C. Winkler (Eds.), John Wiley & Sons, Toronto, Ontario, pp. 1-11.

Winkler, W. G. (1968). Airborne rabies virus isolation. Bull. Wildlife Dis. Assoc. 4:37-40.

Winkler, W. G., E. F. Baker and C. C. Hopkins (1972). An outbreak of non-bite transmitted rabies in a laboratory animal colony. Am. J. Epidemiol. 95:267-277.

Winkler, W. G., T. R. Fashinelt, L. Leffingwell, P. Howard and J. P. Conomy (1973). Airborne rabies transmission in a laboratory worker. J. Am. Med. Assoc. 226:1219-1221.

Wolf, H. W., P. Skally, L. B. Hall, M. M. Harris, H. M. Dicker, L. M. Buchanan and C. M. Dahlgren (1959). Sampling Microbiological Aerosols. Pub. Hlth. Monograph No. 60, U.S. Dept. Hlth. Ed. Welfare, 53 pages.

Wolf, H.W., P. Skally, L.B. Hall, M.M.Harris, H.M.Dicker, L.M. Buchanan, and C.M. Dahlgren (1964). Sampling Microbiological Aerosols. Pub. Hlth. Monograph. No. 60. U.S. Dept. Hlth. Ed. Welfare, 53 pages.

Wolf, L. G., R. A. Griesemer and R. L. Farrel (1968). Experimental aerosol transmission of Yaba virus in monkeys. J. Nat. Cancer Inst. 41:1175-1195.

Wolfe, E.K. (1961). Quantitative characterization of aerosols. Bacteriol.Rev. 25:194-202.

Woode, G.N., and J.C. Bridger (1975). Viral enteritis of calves. Vet. Rec. 103:44-46.

Woode, G.N., J. Bridger, G.A. Hall, J.M. Jones and G. Jackson (1976). The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. Med. Microbiol. 9:203-209.

Woode, G.N. (1982). Rotaviruses in animals. In Virus Infections of the Gastrointestinal tract. D.A.J.Tyrrell and A.Z. Kapikian (Eds.), Marcel Dekker Inc., New York, pp. 295-314.

World Health Organisation (1970). Smallpox in Europe and its aerial spread. Med. J. Aust. 4:861-862.

Wyatt, R.G., W.D. James, E.H. Bohl, K.W. Theil, L.J. Saif, A.R. Kalica, H.B. Greenberg, A.Z. Kapikian, and R.M. Chanock (1980). Human rotavirus type 2: cultivation in vitro. Science 207:189-191.

Yates, W. D. G., K. W. F. Jericho and C. E. Doige (1983). Effect of viral dose on experimental pneumonia caused by aerosol exposure of calves to bovine herpesvirus 1 and Pasteurella haemolytica. Can.

J. Comp. Med. 47:57-63.

Yolken, R. and M. Murphy (1982). Sudden infant death syndrome associated with rotavirus infection. J. Med. Virol. 10:291-296.

Zississ, G., J.P. Lambert, J. Fonteyne and D. De Kegel (1976). Child-mother transmission of rotavirus? Lancet 1:96-98.

Zississ, G., J.P. Lambert, P. Marbehant, D. Marissens, M. Lobmann, P. Charlier, A. Delem, and N. Zygraieh (1983). Protection studies in colostrum-deprived piglets of a bovine rotavirus vaccine candidate using human rotavirus strains for challenge. J. Infect. Dis. 148:1061-1068.