STUDIES ON THE SURVIVAL OF RHINOVIRUS-14 IN AIR AND ON ENVIRONMENTAL SURFACES

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by

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SUMMARY

Human rhinovirus-14 (RV-14) survival in air was studied under varying conditions of relative humidity (RH) and air temperature. The virus, suspended in tryptose phosphate broth (TPB), was aerosolized into a rotating drum using a Collison nebulizer. The virus aerosols were collected with an all-glass impinger. At 20±1°C and high (80±5%) RH the half-life of the virus was 13.7±1.91 h; at the medium (50±5%) and low (20±5%) RH, airborne RV-14 lost its infectivity within 15 min. At 6±1°C and high and medium RH, the half-life of the airborne virus was extended to 38.9±4.01 and 4.09±2.05 h, respectively. However, the lower temperature could not offset the deleterious effects of low RH on virus infectivity.

To study virus survival on environmental surfaces, stainless steel disks were contaminated with RV-14 suspended in either TPB, bovine mucin (BM) or human nasal secretions (NS) and its survival tested at 20°C for 24 h in a humidity-controlled transparent chamber. At the high, medium and low RH, the titre of TPB-suspended virus dropped by <1 $\log_{10}$. With BM as the suspending medium, there was a 0.95, 1.10 and 1.60 $\log_{10}$ drop in virus titre when the RH was at the high, medium and low levels, respectively. RV-14 suspended in NS survived a maximum of 8 hours at high and medium RH, and for no more than 4 h at low RH.

These findings suggest that RV-14 survival in air and on environmental surfaces is affected particularly by RH. However, its potential for survival was greater on surfaces at a wider range of RH tested. Therefore, the results of this study may help to explain the seasonal variations in outbreaks due to rhinoviruses.

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LIST OF ABBREVIATIONS

BM	Bovine mucin
BUDR	5-Bromodeoxyuridine
°C	Degrees centigrade
cm	Centimeter
CPE	Cytopathic effect
CsCl	Cesium chloride
EBSS	Earle's balanced salt solution
FCS	Fetal calf serum
FMDV	Foot-and-Mouth Disease Virus
GMT	Geometric mean titre
H	Hours
IgA	Immunoglobulin class A
IgG	Immunoglobulin class G
Ki	Virus inactivation rate
L	Litre
LVAS	Large Volume Air Sampler
m	Meter
MEM	Minimal essential medium
mg	Milligram
MID	Minimum Infective Dose
mL	Millilitre
nm	Nanometer
NS	Nasal secretions
PBS	Phosphate buffered saline

PFU	Plaque forming units
PSN	Penicillin, Streptomycin, Neomycin
RH	Relative humidity
RNA	Ribonucleic acid
RV	Human rhinovirus
RV-14	Human rhinovirus type 14
SD	Standard deviation
T	Temperature
$TCID_{50}$	Tissue culture infective dose (50%)
TPB	Tryptose phoshpate broth
um	Micrometer

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REVIEW OF THE LITERATURE

INTRODUCTION

Rhinoviruses are small (20-30 nm in diameter), non-enveloped viruses that contain single-stranded RNA. They comprise the largest genus within the family Picornaviridae. Ever since their first isolation in 1956 from nasopharyngeal washings of patients with mild upper respiratory disease (Price, 1956; Pelon et al., 1957), they have been the subject of wide interest . As a result, an ever increasing number of serotypes have been identified (Hamparian et al., 1970; Kapikian et al., 1971; Kapikian, 1972). To date, over 100 distinct serotypes of human rhinoviruses are known to exist (Gwaltney, 1982). However, it is not known if all antigenic types of this virus group have been identified or if new types continually arise due to antigenic drift in nature (Calhoun et al., 1974). Recent reports of intermediate strains or "intertypes", suggest that antigenic drift or genetic recombination may be occurring in rhinoviruses (Halfpap and Cooney, 1983). Some of the biological characteristics of this large virus group are outlined in Table 1.

EPIDEMIOLOGY OF RHINOVIRUS INFECTIONS

Respiratory viruses are probably the most common cause of symptomatic human infections. Among children, respiratory viruses are the major cause of upper as well as lower respiratory tract illness and have been associated on a world-wide basis with significant morbidity, physician visits, hospitalization and death (Glezen and Denny, 1973). In a study of hospitalized children with respiratory disease, it was found that, after respiratory syncytial virus, rhinoviruses were the most commonly isolated agents (Stott et al., 1969). Whereas in adults, infections due to respiratory viruses are relatively benign (Monto and Ullman, 1974), they lead to considerable absenteeism from work and school. A survey

TABLE 1

BASIC BIOLOGICAL CHARACTERISTICS OF RHINOVIRUSES

Feature	Brief Description	Reference
Morphology and Structure	non-enveloped virions, icosahedral symmetry. Capsid composed of 60 identical four-chain polypeptide units.	Macnaughton (1982)
Genome	ss RNA. Positive sense. Genome consists of single molecule of RNA (2.3×10^6 daltons).	"
Virion Protein	Capsids comprise four different structural polypeptides, VP1,VP2,VP3,VP4.	Rossman et al.(1985)
Size	15-30 nm mean diameter of infectious virion varies depending on serotype and method of measurement.	Macnaughton (1982)
Buoyant Density	1.38-1.41 g/cm^3 in cesium chloride.	"
Serotypes	At least 115 serotypes identified, most antigenically distinct. Some cross reactions detected by haemagglutination and neutralization. At least two families of cellular receptors present on human cells.	Hamparian et al.(1970) Macnaughton (1982) Abraham and Colonno, (1984)
Cell Culture Propagation	Rhinoviruses require low temperature and low bicarbonate concentration in order to produce CPE in HEKC (human embryonic kidney cell). Also propagated in HeLa, L-132 and human embryonic lung, WI-38 cells.	Gwaltney (1982)
Host Range	In addition to human, bovine and equine serotypes have also been isolated and produce respiratory tract infections in their respective hosts.	Newmann et al.(1977) Yamashita et al., (1985)
Inactivation and Stability	Stable between pH 6.0 and 8.0, labile at pH <5, in contrast to other enteroviruses. Heat labile above 50°C, addition of $MgCl_2$ stabilises some types. Resistant to lipid solvents such as ether and chloroform.	Macnaughton, (1982)
Transmission	Direct contact via contaminated hands or objects, large droplet, aerosol (?)	Hendley et al.(1973) Gwaltney (1982)

conducted in the U.S. found that such infections, most of which are due to rhinoviruses (Hamparian, 1979), account for a loss of nearly 250 million work days every year; this results in an economic loss of about 5 billion U. S. dollars annually (Merigan, 1982).

As rhinovirus infections occur commonly throughout the world, and are probably the most frequent cause of acute respiratory illness in man, several major epidemiological studies have been done to measure the annual infection rates in different populations (Gwaltney et al., 1966, 1967, 1968; Hendley et al., 1969; Monto and Cavallaro, 1971, 1972; Monto and Johnson, 1967, 1968; Fox et al., 1972, 1975, 1985).

In a three-year prospective epidemiological survey of an industrial population of fifty families in Charlottesville, Virginia, rhinoviruses were isolated from 23% of 1,025 cases of respiratory illness (Gwaltney et al., 1966). Infections due to these viruses occurred throughout the year, being most prevalent during early fall and spring. During the annual fall peaks, rhinovirus accounted for 45% of all cases of respiratory illnesses (Gwaltney et al., 1967). No single serotype was found to predominate during a particular year. An overall infection rate of 0.77 episodes/person/year was observed (Gwaltney et al., 1966, 1967, 1968). The associated illness had a median of 7.4 days and was characterized by rhinorrhea, nasal obstruction, sneezing and, to a lesser extent, cough and hoarseness.

Similar rates of rhinovirus illness were observed in two other major epidemiological surveys. In one prospective study of respiratory illness conducted in Tecumseh, Michigan, approximately 14,600 cases of respiratory illness in 4,905 residents were studied from 1965 to 1971 (Monto and Ullman, 1974). Rhinoviruses accounted for 38.5% of isolates recovered from these illnesses, making them by far the largest proportion

of the isolates. The overall rhinovirus illness rates were 0.29/person/year, with a range of 0.58 for children less than one year old to 0.14 in persons 50 years or older.

The illness rate recorded in the above-mentioned study is probably an under estimation, as similar epidemiological studies have reported higher rates of annual infection (Gwaltney et al., 1966, 1967; Fox et al., 1972, 1985). A possible reason for this discrepancy could be that in this study, telephone contact was used to determine the presence of illness; whereas, in other studies, such as the Seattle Virus Watch (Fox et al., 1972), a nurse epidemiologist paid periodic visits to the house-holds being surveyed, resulting in reported illness rates of 0.64/person/year. As was observed earlier in the Seattle Virus Watch (Fox et al., 1972, 1975), the Tecumseh study also noted the highest rhinovirus infection rates in infants and school-age children, indicating the importance of this age group in the spread of rhinovirus colds within the general population (Monto and Ullman, 1974).

Extending the original Seattle Virus Watch of 1965-69 (Fox et al., 1972), a further 4-year prospective epidemiological study was carried out from 1975-79 (Fox et al., 1985). Again, two seasonal peaks of illness (one in fall and the other in spring) were evident; this time the spring break was the more predominant. A total of 96 different rhinovirus serotypes were recovered during the entire observation period 1966 to 1979. No single serotype appeared to predominate, but certain serotypes seemed to persist and were isolated more frequently during certain periods.

The above-mentioned epidemiological studies as well as others (Hamre et al., 1966; Spigland et al., 1966) have documented increase in cases of

rhinovirus infections that occurr in early fall and late spring. Furthermore, it has been observed that in the tropics, rhinovirus infections peak during the rainy season (Monto and Johnson, 1967, 1968).

In an attempt to explain the seasonality of rhinovirus colds, it was postulated by some that the early fall peaks of such illness might be associated with school openings at least in the temperate regions. Hendley et al. (1969), however, found no apparent association between school openings and peak rhinovirus illness rates in working adults with or without school-age children. Although the seasonality of rhinovirus infections may be related to certain environmental and metereological factors, no published information in this regard is as yet available.

In summary, rhinoviruses have been found to be associated with 20 to over 40% of all cases of acute respiratory illness in adults and children. The susceptibility to rhinovirus illness generally decreased with increase in the age of an individual. Different annual infection rates for adults and children have been observed by different investigators. These observations may be due to differences in reporting , age distribution of populations surveyed, criteria for defining the illness and variations in the sensitivity of the diagnostic methods employed. There is a marked seasonal variation in rhinovirus infection rates, with annual epidemics in the early fall and spring. The reasons for his seasonality remain as yet unexplained.

IMMUNITY

The presence of serum as well as nasal secretory antibodies has been associated with type specific resistance to illness following natural or experimental exposure to rhinoviruses (Cate et al., 1966; Hendley et al., 1972).

Titers of rhinovirus-neutralizing antibody in the serum rise in 70-80% of experimentally- or naturally-infected individuals, and seem to persist for many years (Hendley et al., 1969, 1972). Type-specific nasal secretory antibody titers were also found to rise in up to 70% of volunteers infected with rhinoviruses, but this type of antibody (IgA) was short-lived when compared to that in the serum (IgG) (Cate et al., 1966). Other studies have also shown that the level of rhinovirus-neutralizing antibody in the nasal secretions begins to decline within a period of five months (Taylor-Robinson, 1963; Holmes et al., 1976b). These findings may explain in part the high rate of recurrent infections observed with rhinoviruses.

In an attempt to determine which type of neutralizing antibody (serum or nasal) is more important in protection against rhinoviral infections, formalin-inactivated rhinovirus-13 (RV-13) was given to two groups of antibody-free volunteers (Perkins et al., 1969a). One group was given the antigen parenterally to induce a serum antibody response; the second group received the antigen intranasally to generate secretory antibody. A month later, the volunteers were challenged intranasally with a standard suspension of RV-13 ($10^{\emptyset}$ $TCID_{50}$). It was found that the intranasal vaccinees exhibited resistance to both infection and illness, whereas the parenteral vaccinees who elicited a solely serum antibody-related response were not protected.

The age-related prevalence of serum neutralizing antibodies to 56 rhinovirus serotypes was tested (Hamparian et al., 1970). The level of antibodies was found to increase with age and then stabilize through adulthood. Serum antibodies to certain rhinovirus serotypes were more prevalent than to other types in the population tested. For example, over 75% of the 148 sera tested had antibody against type 49, whereas

less than 10% of the samples tested could neutralize type 7. This may be because certain rhinovirus serotypes have a greater virulence, higher antigenicity or transmissibility in the general population. The observed difference in the prevalence of type-specific neutralizing antibodies may also represent a heterotypic antibody response. The importance of heterotypic antibody responses and cross reactions in immunity to rhinoviruses is still not clearly understood. However, certain rhinovirus serotypes have been found to illicit heterotypic responses when tested in adult volunteers, whereas other serotypes could not do so (Fleet et al., 1968).

Very few studies have been done to elucidate the role of cellular immunity in protection against rhinovirus infections (Turner and Gwaltney, 1984). It has recently been shown that polymorphonuclear leukocytes (PMN) migrate into the nasal mucosa of individuals with rhinovirus colds (Winther et al., 1984). The role of these cells in the defence against rhinoviruses is as yet unclear. Other studies have detected interferon in respiratory secretions during the course of experimental rhinovirus infections (Cate et al., 1969).

PATHOGENESIS OF RHINOVIRUS INFECTIONS

The exact mechanism by which rhinoviruses produce rhinitis and other disease symptoms is unknown. The acute stage of rhinoviral rhinitis is characterized by edema of the nasal mucosa with exudation of serous and mucinous fluid. At this stage of the infection, epithelial cell scrapings from the intranasal passages were found to be uniformly positive for rhinovirus antigens when tested by immunofluorescence or immunoperoxidase staining; however, nasal biopsies of rhinovirus-infected individuals showed no cellular pathology of the epithelial, or subepithelial layers (Douglas, 1970; Turner et al., 1982; Turner and

Gwaltney, 1984). Histamine levels were not found to increase during rhinovirus colds, and the number of mast cells in the nasal mucosa remained unaltered (Winther et al., 1984). Although it has been suggested that a number of inflammatory substances such as prostaglandins, kinins and leukotriens may be involved in the pathogenesis of rhinovirus infection, no studies have thus far been carried out in this regard.

The incubation period of rhinovirus infections is usually 2 to 3 days, and the onset of illness is related to the time of peak virus shedding (Cate, 1973; Douglas et al., 1966).

Since rhinoviruses have been isolated in high titers from nasal and oropharyngeal secretions, it has been accepted that the nasopharynx is the primary site of virus replication; however, the exact anatomical limits of rhinovirus infection have not been determined. A large percentage of patients infected with rhinoviruses show upper as well as lower respiratory tract symptoms (Cate et al., 1965; Couch et al., 1966; Bush et al., 1978; Halperin et al., 1983), suggesting infection of the lower respiratory tract as well. Rhinoviruses have also been recovered from the sputum of asthmatic children (Horn et al., 1979; Minor et al., 1976) and from adults with chronic bronchitis (Lambert and Stern, 1972).

In a more recent attempt to investigate the pathogenesis of lower respiratory tract symptoms in experimental rhinovirus infection, susceptible volunteers were inoculated intranasaly with a standard dose of rhinovirus and then specimens were obtained by bronchoscopy at the time of peak infection (Halperin et al., 1983). Infectious rhinovirus particles were isolated from bronchial wash specimens of 5 of 13 infected volunteers in titers similar to those found in nasal secretions. This finding strongly suggests direct infection of the lower respiratory tract

with rhinoviruses. It is possible that the number of virus-positive samples from the lower respiratory tract would be higher if the volunteers were challenged with viral aerosols suitable for retention in bronchi and alveoli. In earlier studies with susceptible volunteers, when aerosol inoculation of rhinoviruses was used, more lower respiratory tract symptoms could be observed compared with when the virus was inoculated by the intranasal route (Cate et al., 1965; Couch et al., 1966).

TRANSMISSION OF RHINOVIRUS INFECTIONS

Systematic studies on the transmission of the common cold through the nasal secretions of infected individuals were first conducted at the Common Cold Research Unit, Salisbury, England (Andrewes et al., 1951; Tyrrell et al., 1960a, 1960b, 1962 ; Tyrrell, 1965). However, these investigations were performed prior to the isolation and characterization of the viruses responsible for causing the common cold, hence the immune status of the volunteers used in these experiments could not be determined. The subsequent discovery and successful in vitro cultivation of human rhinoviruses made it possible to confirm and further extend the initial observations of Andrewes, Tyrrell and their co-workers.

Rhinovirus Shedding: Rhinovirus shedding, the first step in the sequence of transmission, occurs primarily from the nose (Table 2). It is known that rhinoviruses are uniformly present in nasal secretions in titers reaching 1,600 $TCID_{50}$/mL and that virus shedding may continue for up to 21 days after infection (Douglas et al., 1966; Fox et al, 1985). About 1.5% of the infected individuals have been found to shed these viruses up to 55 days following infection (Fox et al., 1985). This prolonged virus shedding can potentiate environmental contamination.

TABLE 2

ESTIMATED FREQUENCY OF RHINOVIRUS SHEDDING ON THE THIRD DAY OF INFECTION

Source	Frequency*	GMT** $TCID_{50}$	Reference
Nasal secretions (wash or swab)	>90%	300	Hendley et al.(1973)
Pharyngeal secretions (gargle and swab)	70%	30	"
Saliva	50%	10	"
Lower Respiratory Tract (bronchoscopy)	46%	100	Halperin et al.(1983)
Aerosol (LVAS, cough/ sneeze plate)	0 and 8%	not done	Couch et al.(1966) Gwaltney et al.(1978)
Hands	50%	10	Gwaltney et al.(1978)
Environment	15%	1	Reed (1975)

*Percent of time virus recovered
**Geometric Mean Titer

Considerably lower titers of infectious rhinovirus particles have been detected in pharyngeal secretions and saliva (Hendley et al., 1973; Gwaltney et al., 1978).

Modes of Transmission: From epidemiological studies it is known that most infections are acquired in the home (D'Alessio et al., 1976, 1984; Meschievitz et al., 1984), and that school-age children have higher rates of rhinovirus infection and are, therefore, considered as important reservoirs of these viruses (Gwaltney et al., 1966, 1968; Fox et al., 1972, 1985).

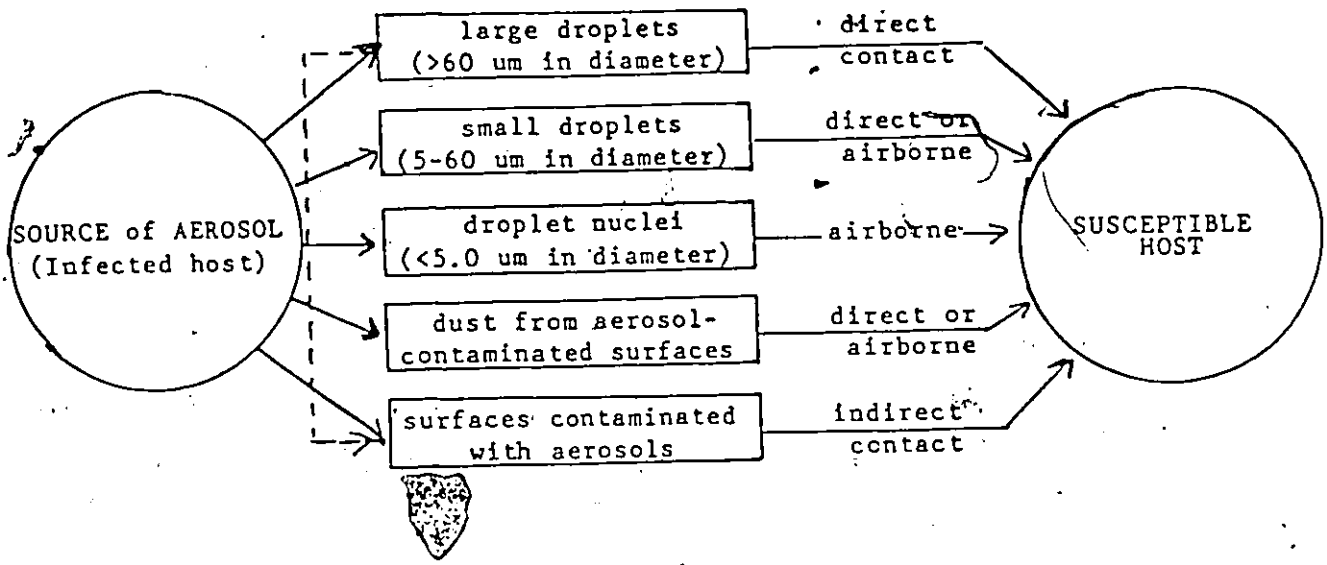
The following two modes of transmission are believed to operate in the spread of rhinovirus colds in nature:

(1) The aerial route, where infectious airborne particles (<5 um in diameter) generated by an infected individual during coughing, sneezing or nose-blowing are inhaled by susceptible hosts.

(2) Person-to-person contact, either direct (such as hand-shaking or kissing) or indirect where self-inoculation follows contact with virus-contaminated fomites or environmental surfaces. Inhalation of large-particle aerosols of nasal secretions by susceptible individuals in the immediate vicinity of rhinovirus-infected persons is considered to be a form of direct person-to-person contact. Fig. 1 summarizes the major pathways for the spread of rhinovirus infections.

The small-particle aerosols or 'droplet nuclei', which may play a role in the aerial spread of rhinovirus colds, can remain airborne for several hours in the indoor environment (Knight, 1973, 1980). When inhaled, they are retained primarily in the lower respiratory tract (Hatch, 1961; Cate et al., 1965; Couch et al., 1966). Coarse droplets of nasal secretions, which are larger than 10 um in diameter, fall to the ground within a matter of minutes (Couch et al., 1966; Gwaltney and Hendley, 1978).

Figure 1. Possible pathways in the spread of rhinovirus infections. (adapted from C.M. Johnson- Lussenburg, personal communication).


SOURCE of AEROSOL
(Infected host)
large droplets
(>60 um in diameter)
small droplets
(5-60 um in diameter)
droplet nuclei
(<5.0 um in diameter)
dust from aerosol-
contaminated surfaces
surfaces contaminated
with aerosols
direct
contact
direct or
airborne
airborne
direct or
airborne
indirect
contact
SUSCEPTIBLE
HOST

Apart from their significance in direct person-to-person spread, these larger particles can effectively deposit rhinovirus-containing secretions in the immediate surroundings of an infected individual.

It has been shown that coughing generates mainly small-particle aerosols derived from secretions in the pharynx and the lower respiratory tract; on the other hand, sneezing and nose-blowing produce a larger proportion of coarse droplets from saliva and nasal secretions, respectively (Gwaltney , 1980).

Minimal Infective Dose: Couch et al., (1966) have demonstrated that as little as 1 $TCID_{50}$ of a given rhinovirus can efficiently produce infection when deposited in the nose of volunteers free from detectable circulating antibodies. They compared this method of inoculation with small-particle aerosol challenge and found that the latter required nearly 20 times the amount of virus to infect susceptible hosts. This suggested that the nasal mucosa is more susceptible to rhinoviruses than the lower respiratory tract. Also, volunteers receiving the nasal inoculation showed typical rhinovirus rhinitis, whereas those given the aerosol challenge had a predominance of tracheobronchitis and other symptoms of lower respiratory tract involvement. This is probably due to the fact that small-particle aerosols are retained largely in the bronchus and the pulmonary lobules (Cate et al., 1965).

Evidence for Direct Contact Transmission: As rhinoviruses cause infections of the respiratory tract, they were traditionaly thought to spread primarily by the airborne route (Cate, 1973). The efficiency of infection initiated by the nasal route as well as the earlier demonstration that infection could be readily contracted by the conjunctival route (Bynoe et al., 1961), prompted studies into the possible role of direct contact in the spread of rhinovirus infections.

Experimental transmission of rhinovirus colds via contaminated hands or objects was first demonstrated by Hendley et al. (1973). In that study, four of eleven susceptible volunteers became infected after touching their nasal or conjunctival mucosa with fingers previously contaminated by rubbing a dried drop of rhinovirus type 39. In agreement with the earlier observations of Bynoe et al. (1961), six susceptible volunteers given the virus by the oral route did not develop infection (Hendley et al., 1973). In a more recent transmission study, kissing between rhinovirus-infected and susceptible volunteers failed to spread the infection (D'Alessio et al., 1984).

Table 3 compares the efficiency of the different routes of transmission in various experimental studies which have been designed to simulate natural transmission. The most efficient mode of transfer of infection was hand-to-hand contact, having an efficiency rate of 73% (Gwaltney et al., 1978).

Rhinovirus Survival on Animate and Inanimate Surfaces: Survival of rhinoviruses on contaminated surfaces is necessary if such surfaces are to act as vehicles for the infections caused by these agents. Following is a summary of what is known about rhinovirus survival on various types of surfaces and the influence of different factors in promoting or retarding such survival.

Rhinovirus type 39 was found to retain its infectivity in nasal mucus for periods up to 24 hours (Hendley et al., 1973). In this study, four of ten persons with rhinovirus-induced rhinitis were found to have detectable levels of infectious rhinovirus on their hands. It was further demonstrated that, under conditions of non-use, RV-13 dried on the skin surface of hands survived up to 3 h. The virus could also survive for at

least three hours on experimentally-contaminated non-porous inanimate surfaces, such as formica, stainless steel, nylon and plastic (Table 4). In contrast to this, virus infectivity was rapidly lost when it was placed on porous materials such as facial tissues and cotton towels. In the same series of experiments, RV-13 was recovered from the fingers in 15 out of 16 trials in which plastic contaminated with the virus one to three hours previously was rubbed by a volunteer. Skin-to-skin transfer of the virus could also be demonstrated in three of five subject pairs.

Reed (1975) could recover RV-2 from the fingers of 16 out of 38 volunteers (42%) who were swabbed during the acute stage of their colds. The virus could also be transferred from surface to surface by rubbing, the transfer being more efficient if it was carried out while the inoculum was still damp. RV-2 was found to survive for several days on stainless steel spoons which were contaminated with the virus and left on a laboratory bench to dry (Reed, 1975).

Self inoculation with RV-2 was shown to occur when a finger contaminated with a moderately heavy dose (88 $TCID_{50}$) of the virus was rubbed into the conjunctivae or the nostrils (Reed, 1975). In the same study, RV-2 was rubbed on smooth surfaces such as table tops, pens and stainless steel objects, which were then handled normally or rubbed with a finger, and transfer of the virus to the recipient finger was found to occur in 64% of the cases.

It should be noted here that in these studies by Hendley et al. (1973) and Reed (1975) no attempts were made to study the effect of air temperature, relative humidity and virus suspending medium on rhinovirus survival on animate and inanimate surfaces.

In a more recent study, Reagan et al. (1981) investigated the effect of air temperature (6, 23 and 37°C) and various suspending media on the

TABLE 3

EFFICIENCY OF TRANSMISSION

Route	Transmission rate	Reference
Combined airborne and large particle aerosol	8%	Gwaltney et al. (1978)
Hand-to-hand contact	73%	"
Kissing for one minute	8%	D'Alessio et al.(1984)

TABLE 4

SURVIVAL OF PICORNAVIRUSES ON SURFACES

REFERENCE	VIRUS	SUSPENDING MEDIA	ENVIRONMENT RH (%)	ENVIRONMENT TEMP (°C)	SURFACE	SURVIVAL	COMMENTS
Hendley et al. (1973)	RV-(39)	Tissue culture& nasal secretions	ambient	23	formica, stainless steel, wood, different fabrics, cotton	>3 hrs.	RV-39 survived better in diluted mucus (24 hrs.) than in undiluted mucus (5 hrs).
					rayon wool	1 hr.	
					paper towel	1 hr.	
					facial tissue	"	
					plastic	72 hrs.	
					skin	3 hrs.	
Reed (1975)	RV-(2)	Tissue culture& nasal wash (mixture of nasal secretions in PBS and bacteriological nutrient broth).	ambient	room	stainless steel spoons,	7 days	
					ball point pens,	24 hrs.	
					plastic nylon	24 hrs.	
					skin	3 hrs.	
Reagan et al. (1981)	RV-(2) & RV-(14)	Tris buffer saline BSA	ambient	6 23 37	polyethylene vials	24 hrs.	BSA and saline had protective effect on virus survival. Inverse relation between virus survival & temperature.

TABLE 4 (continued).

TABLE 4

REFERENCE	VIRUS	SUSPENDING MEDIA	ENVIRONMENT RH (%)	ENVIRONMENT TEMP (°C)	SURFACE	SURVIVAL	COMMENTS
Dixon et al. (1966)	Polio (2)	Tissue culture	35 78	25	cotton wool	1-4 wks. 20 wks.	At 78% RH the decrease in virus titer was less rapid than at 35% RH.
Mahl& Sadler (1975)	Cox-sackie B3	Tissue culture	3 55 96	25 37	glass slides	2 wks.	Differences in survival due to temp. or RH not detected.
Mahl& Sadler (1975)	Polio (2)	"	"		"	8 wks.	
McGeady et al. (1979)	Cox-sackie B3	Tris buffer saline BSA	ambient	6 23 37	polyethylene vials	24 hrs.	BSA had protective effect upon virus survival. Inverse relation between virus survival & temperature.

capacity of RV-2 and RV-14 to survive on polyethylene vials. An inverse relationship between virus survival and air temperature was observed and the survival patterns of both the serotypes were found to be very similar. Among the suspending media tested in this study, bovine serum albumin and normal saline were found to protect virus infectivity better than tris-buffer.

Some work has been done on the survival of other picornaviruses on inanimate surfaces (Dixon et al., 1966; Mahl and Sadler, 1975; McGeady et al., 1979). The findings of these studies are also summarized in Table 4.

Evidence for Airborne Transmission: Whereas susceptible volunteers have been successfully infected by exposure to small-particle aerosols of rhinoviruses, it has been extremely difficult to document airborne transmission of these viruses under natural conditions. In experimental studies, rhinoviruses have not readily spread from person to person by the airborne route (Gwaltney et al., 1978; D'Alessio et al., 1984). Attempts to recover infectious rhinoviruses in small-particle aerosol by employing a large volume air sampler have also been unsuccessful (Couch et al., 1966). However, earlier work in our laboratory has shown that infectious RV-14 in artificially-generated aerosols (<5 um in diameter) could be recovered by a prototype large volume air sampler (Park, 1980).

In one transmission experiment designed to simulate large-particle aerosol exposure, susceptible individuals seated around a table with rhinovirus-infected subjects who sang, laughed and talked loudly did not become infected after 15 minutes of contact with recipients (Gwaltney et al., 1978). In this case both donors and recipients wore rubber gloves in the experimentation room. In other experiments, susceptible volunteers, separated by a double wire-mesh to preclude any direct physical

contact with infected subjects, did not become infected, even after three consecutive days and nights of exposure (Gwaltney et al., 1978). In a similarly designed airborne transmission study of RV-55, infected and susceptible volunteers were housed together in a small dormitory room for a period of twelve hours a day for three consecutive days. Individuals were instructed to keep all doors and windows shut during the experiment and to avoid handling one another's personal items. Out of a total of ten susceptible volunteers, only one case of RV-55 transmission could be demonstrated (D'Alessio et al., 1984).

The efficacy of all three routes of transmission (i.e. direct contact, large- and smal-particle aerosols) was compared extensively (Gwaltney et al., 1978; Gwaltney and Hendley, 1978). It was found that transmission of infection was very efficient by the hand contact route; 11 of 15 hand contact exposures between infected and susceptible volunteers initiated infection, compared with only 1 of 12 large-particle and none of small-particle aerosol exposures.

Infectious rhinovirus particles could not be readily recovered from cough and sneeze specimens of infected volunteers using the petri dish collection method (Hendley et al., 1973). This finding has been used to further discount airborne transmission of rhinoviruses. In this method, secretions from a simulated cough or sneeze are collected in a glass petri dish coated with collecting broth and held 8 cm from the face of the test subject. We know that the virus does survive the process of aerosolization (Couch et al., 1966) and has been detected in oropharyngeal secretions as well as in saliva and therefore should have been detected in coughs and sneezes. The above mentioned method used for detection seems to be an insensitive one. Couch et al., (1970), were readily able to recover another picornavirus, Coxsackie A type 21 from

both coughs and sneezes using a balloon collection method. This collection method involves coughing or sneeezing into a collapsed weather balloon through a tight fitted face mask to minimize viral loss. The air in the balloon is then evacuated through a Shipe impinger to remove airborne particles, and material impacted on the inside wall of the balloon is collected by rinsing with cell culture fluid. It might have been possible to detect virus in cough and sneeze specimens had Hendley et al., (1973) used this collection method.

In conclusion, it seems that both direct as well as indirect contact are important mechanisms of rhinovirus transmission under natural conditions. Airborne transmission, although difficult to demonstrate experimentally, cannot be discounted and probably plays a role in conjunction with direct contact transmission.

As pointed out previously for the surface studies, investigators testing the possible airborne transmission of rhinoviruses did not make any specific mention of the temperature and relative humidity of the air within the test environment. It is conceivable that under the particular indoor conditions in which these experiments were carried out, the virus did not survive long enough in the air to permit its transmission.

Survival of Airborne Rhinoviruses: No published information is as yet available on how various atmospheric factors, such as air temperature and relative humidity, affect the survival of airborne rhinoviruses. Studies with another closely related picornavirus, foot-and-mouth disease virus (FMDV), have shown that this virus survives best in air at high (>80%) relative humidity and is rapidly inactivated at low (<50%) relative humidity levels (Donaldson, 1972; Gloster, 1983). Similar findings have been reported for polioviruses (Harper, 1961, 1963; Ijaz et al., 1985b).

RHINOVIRUS PREVENTION AND CONTROL

In the earlier days of research into the common cold, great emphasis and efforts were placed on developing a vaccine against rhinoviruses (Fleet et al., 1965; Cate et al., 1966; Perkins et al., 1969b; Buscho, 1972). A major obstacle has of course been the multitude of serotypes encountered in nature (Fox, 1976). As has been discussed, there is also the problem of inducing long lasting nasal mucosal immunity. Conventional parenteral vaccination with inactivated rhinoviruses has been shown to be ineffective (Cate et al., 1966; Douglas and Couch, 1972). Live attenuated, or inactivated intranasal vaccines have as yet not been developed. The lack of sufficient knowledge of rhinovirus molecular biology, and some of the problems cited above have seriously discouraged vaccine development. In fact, at the present time all efforts into developing rhinovirus vaccines have been abandoned, and more emphasis is now being placed on the interruption of transmission as a method of control of the spread of these viruses (Couch, 1984).

Controlling the Spread of Rhinoviruses: Apart from frequent hand-washing, the use of disinfectant-impregnated handkerchiefs has been shown to be quite effective in interrupting rhinovirus transmission. An iodine-incorporated handkerchief was tested in Antarctica, and was found to reduce the incidence of colds (Dick et al., 1980). However, the odor and staining properties of iodine kept these handkerchiefs from becoming more widely accepted. In a more recent attempt, the high acid lability of rhinoviruses has been exploited to develop a new kind of disinfecting handkerchief (Holz et al., 1984; Kuhrt et al., 1984). Citric acid alone or a combination of citric and maleic acids (at a pH of 2.7) has been found to be an efficient, cheap and safe virucide against rhinoviruses.

When incorporated into paper handkerchiefs, one square inch could inactivate at least 10^6 infectious rhinovirus particles (Dick et al., 1986). In order to deal with the enveloped viruses (e.g. corona- and paramyxoviruses) known to cause the common cold, sodium lauryl sulfate has also been added to these handkerchiefs.

These disinfectant-treated handkerchiefs have now been tested in an experimental setting to see if they could interrupt the transmission of rhinovirus colds (Dick et al., 1986). In this experiment, volunteers infected with RV-16 were seated around tables together with susceptible volunteers. They were then to engage in a rowdy poker game for 12 hours, so as to try to simulate all possible modes of transmission (i.e. direct, indirect contact as well as large and small-particle aerosols). One group of infected and susceptible volunteers was provided with the virucidal handkerchiefs, whereas the control group was allowed to use only the ordinary cotton handkerchiefs. None of the susceptible individuals using the virucidal handkerchiefs contracted the infection, but 42-75% of those using the ordinary handkerchiefs came down with the common cold.

Treatment of Rhinovirus Infections: Daily intranasal administration of interferon alpha-2 for a trial period of three weeks was found to protect against rhinovirus infections when tested in a group of volunteers (Farr et al., 1984). However, after this trial period a considerable percentage of the volunteers developed nasal irritation, obstruction and bleeding. In recent randomized, double blind, placebo-controlled trials only short-term treatment with intranasal interferon alpha-2 as 'contact prophylaxis' was provided whenever the individual was exposed to a person with a cold in a family setting (Douglas et al., 1986; Hayden et al., 1986). In these studies, treatment periods were limited to only a few days and when used in this way, alpha-2 interferon was well

tolerated and nasal symptoms were minimized. This post-exposure prophylaxis with intranasal interferon proved to be an effective strategy in the prevention of spread of natural colds caused by rhinoviruses. Unfortunately, this treatment seemed to be less efficacious in the prevention of colds due to other types of viruses (Douglas et al., 1986).

Other antiviral substances such as enviroxime (2 amino-1-isopropyl sulphonyl-6-benzimidazole phenyl ketone oxime) which is a strong inhibitor of rhinovirus replication in vitro, has been found to be totally ineffective in the prevention and treatment of infections due to these viruses (Miller et al., 1985).

MAIN OBJECTIVES OF THE STUDY

1. To compare the suitability of L-132 and A-5 HeLa cell lines for the cultivation and plaque assay of RV-14.
2. To assess the capacity of RV-14 to survive in the airborne state under varying conditions of relative humidity and atmospheric temperature.
3. To study the survival of RV-14 on stainless steel surfaces under varying conditions of relative humidity at ambient temperature.
4. To determine the effect of different virus suspending media on the survival of RV-14 on experimentally-contaminated surfaces.
5. To investigate the correlation, if any, between the airborne survival of RV-14 versus its survival pattern on surfaces under the same environmental conditions of relative humidity and temperature.
6. To discuss the above findings in relation to virus transmission and seasonality of outbreaks of infections due to rhinoviruses.

MATERIALS AND METHODS

CELLS AND VIRUS

A-5 HeLa Cells: The A-5 strain of HeLa cells was used for rhinovirus propagation and quantitation. A seed culture of these cells was kindly provided to us by Dr. B. Korant (E.I. du Pont de Nemours and Co., Willmington, Delaware, U.S.A). They were routinely grown in Eagle's minimal essential medium (MEM "Auto-Pow', Flow Laboratories Inc., Mississauga, Ont.). Five hundred mL of the medium was supplemented with 5.0 mL of 200 mM L-glutamine (GIBCO, Burlington, Ont.), 13.4 mL 20 mM sodium bicarbonate (Fisher Scientific, Fair Lawn, N.J., U.S.A.) and 0.5 mL of an antibiotic mixture (PSN) to give a final concentration of 100 ug/mL of penicillin (P) and streptomycin (S) and 50 ug/mL of neomycin (N). The medium was further supplemented with 50 mL of heat-inactivated (56°C, 30 min) fetal calf serum (FCS; Flow Labs.). The cell monolayers were passaged at a split ratio of 1:3 every two days and grown at 37°C in 80 cm^2 disposable plastic flasks (Nunc Inc., Burlington, Ont.). They were generally ready for use within 48 hours of seeding.

L-132 Cells: This is a continuous line of human lung cells. A seed culture of these cells was originally obtained by us from Mr. D.A. McLeod of the Laboratory Centre for Disease Control (L.C.D.C.) Ottawa, Ontario. These cells were passaged at two-day intervals at a split ratio of 1:3. and were maintained between passages 20 and 40 (from the date of introduction in our laboratory) using the same growth medium and flasks as for the A-5 HeLa cells. They were used within 24 hours of reaching confluence.

Rhinovirus: Human Rhinovirus type 14 (RV-14) was obtained through the courtesy of Dr. J. Gwaltney, Jr. (University of Virginia, Charlottesville, VA, U.S.A.). The virus was plaque purified once in our laboratory using A-5 HeLa cells. For the preparation of the virus pools,

each 80 cm^2 flask with a confluent monolayer of the cells received 0.5 mL of the inoculum to give a multiplicity of infection of about 1:100, and the monolayers were maintained in MEM containing 2% FCS. Within 48 hours of incubation at 33°C, nearly all the cells in the monolayer showed virus-induced cytopathic effects (CPE). The infected cultures were then frozen (-80°C) and thawed three times and the cell culture harvest was centrifuged at 3,000 rpm for 15 min. The supernatant containing the virus was then diluted 1:10 either in tryptose phosphate broth (TPB; Difco, Detroit, MI, U.S.A.) reconstituted bovine mucin, 5 mg/mL (Sigma, St. Louis, MO., U.S.A.) or nasal secretions before being stored at -80°C.

Radiolabelling of Rhinovirus-14: Growth medium was discarded from monolayers of A-5 HeLa cells and they were washed twice with Earle's balanced salt solution (EBSS). The cells were then 'starved' in EBSS without serum for 12 hours before being infected with the virus at a multiplicity of infection of about 1:100. The virus was allowed to adsorb for 2 hours at 33°C. At the end of this period, 12 mL of radiolabelling medium was added to each monolyer. This medium consisted of EBSS supplemented with 1/20th the normal MEM amino acid concentrations, 1.0 uCi/mL of L-[U-^{14}C]-labelled amino acids (Amersham, Oakville, Ont.), 30 mM $MgCl_2$. After 72 hours at 33°C, a three-plus CPE could be observed. The virus was separated from cell debris as described above. The supernatant was then resuspended in a 40% (w/v) solution of cesium chloride (CsCl) and subsequently layered onto a 55% (w/v) solution of CsCl and then subjected to density gradient ultracentrifugation at 132,000g for 20 hours. The fractions were collected from the bottom of the centrifuge tube and their refractive index and radioactivity measured. Those fractions corresponding to the density of complete rhinovirus particles,

1.38g/cm^3, were pooled and dialysed to remove unincorporated ^{14}C-labelled material. Plaque titers of the radio-labelled virus were found to be similar to those of the regular virus pools.

Rhinovirus Antiserum: Hyperimmune monkey antiserum against RV-14 (strain UP3, 1059) was kindly provided to us by Dr. J. Gwaltney, Jr. In a neutralization test to confirm the serotype identity of RV-14, this antiserum completely inhibited rhinovirus plaque formation at a dilution of 1:1000, when the amount of challenge virus was 400 PFU/mL.

Rhinovirus Plaque Assays: For the plaque assay of RV-14 (Fiala, 1968) each monolayer of HeLa cells in 80 cm^2 flasks was inoculated with 0.33 mL of the material to be quantitated, using at least 3 monolayers for each dilution. The control cultures received the same quantity of the growth medium. After one hour of virus adsorption at 33°C, each culture was overlaid with 25 mL of medium M-199 (Flow Labs.), supplemented with 0.22% $NaHCO_3$, 100 ug/mL of 5-bromodeoxyuridine (BUDR; Calbiochem., San Diego, CA., U.S.A.), 50 ug/mL DEAE-dextran (Sigma), 30 mM $MgCl_2$ and 0.9% Oxoid Agar No. 1 (Oxoid, Hampshire, England). The cultures were then placed back at 33°C. Virus plaques were generally ready for counting within 72 hours, at which time each culture received 5 mL of a 4% formol-saline solution and fixation was allowed to proceed for 24 hours at room temperature. The agar layer was then removed and the fixed monolayers were then throughly washed in tap water. They were subsequently stained with a 1% aqueous solution of crystal violet.

Virus Suspending Media: In all of the aerobiological experiments, TPB was used as a suspending medium for the generation and collection of RV-14 aerosols. In preliminary tests, TPB was found to be completely harmless to the infectivity of this virus. Morever, TPB, as opposed to the use of MEM, was considered to simulate more closely the composition

of body secretions. By using TPB as a suspending medium, we could also make closer comparisons between the patterns of survival of airborne RV-14 and the other viruses previously studied in our laboratory (Ijaz et al., 1985a, 1985b; Sattar et al.; 1984).

In the surface survival experiments, three different suspending media were tested: TPB, bovine mucin and human nasal secretions. Mucin solution was made up at a concentration of 5mg/mL in physiological saline, which represented the upper limit of normal concentrations of mucin in human nasopharyngeal secretions (Documenta Geigy, 1970). The nasal secretions were collected during the acute stages of a cold from an adult male volunteer. These secretions were first filtered through a Millipore membrane filter (0.22 um pore diameter) and the filtrate inoculated into monolayers of both A-5 HeLa and L-132 cells. No apparent cytopathology was observed even after five days of incubation (33°C) of these cultures under either a liquid or agar overlay. The pH of the nasal secretions was tested and found to be neutral.

EQUIPMENT AND TEST PROCEDURES FOR AEROBIOLOGY EXPERIMENTS

Nebulizer: Aerosols of RV-14 were generated by a 6-jet Collison nebulizer (BGI Inc., Waltham, MA, U.S.A.). This nebulizer was chosen because >90% of the aerosol particles produced by it are <5 um in diameter. They represent particles capable of retention in the respiratory tract (May, 1973). Morever, a certain proportion of the airborne particles generated during the process of sneezing and coughing also fall in this size range and they are believed to play a role in the airborne spread of the common cold in nature (Couch et al., 1966).

The Rotating Drum: A 300-L stainless steel toroidal drum was used for the storage of the viral aerosols (Goldberg et al., 1958). It was housed

in an insulated temperature-controlled cabinet (Fig. 2). The drum was rotated at 4 rpm to reduce the settling of the aerosols. The drum was cleaned by flushing it with air (at a pressure of 1.8 kg/cm^2) for four hours to get rid of any residual virus or dye that might be left between experiments.

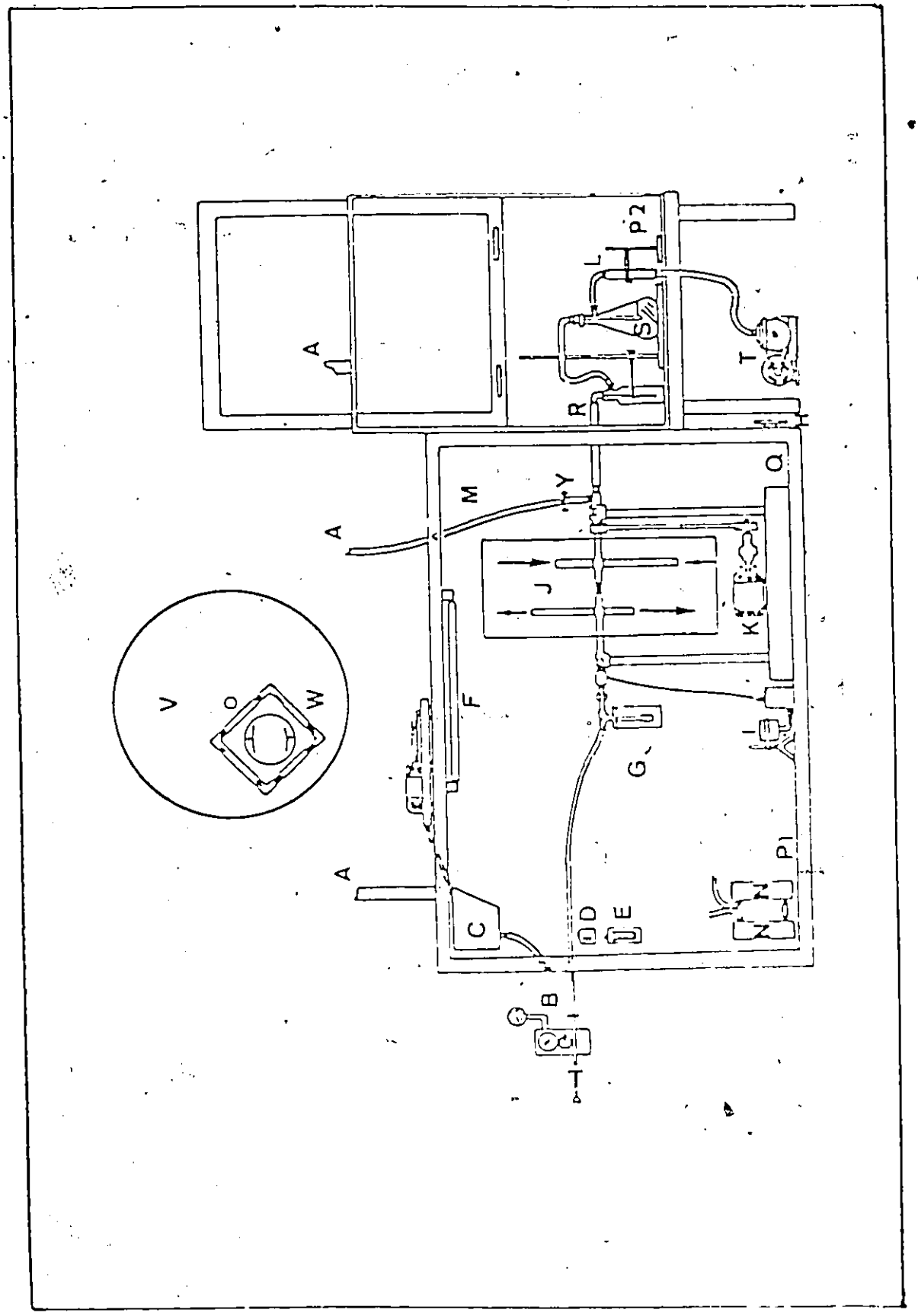
Impingers: Viral aerosols were collected using the all-glass impinger (Wolf et al., 1964) purchased from BGI Inc. To trap virus particles more efficiently, these impingers were modified by us to give a distance of 15 mm between the tip of the limiting orifice and the surface of the collecting fluid (10 mL) in the impinger. A critical vacuum was maintained in all experiments so that the impinger would operate at its design capacity of 5.6 L/min. Between experiments, the impingers were decontaminated by autoclaving, cleaned by sonication in 7x detergent solution (Flow Labs.), washed thoroughly and then autoclave sterilized.

Adjustment of Relative Humidity and Temperature in the Rotating Drum: Virus survival was tested at the low (20.5%), medium (50±5%) and high (80±5%) levels of relative humidity (RH). All RH measurements were made with the aid of a dial-type 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.

To achieve the low RH level, the drum was filled with air passed through a Drierite cylinder (Hammond Drierite Co., Xenia, OH, U.S.A.) prior to nebulizing the virus. To obtain the medium or the high RH level, distilled water was sprayed into the drum until the desired RH level was reached. Following an equilibration period of several hours, the virus was aerosolized into the drum. The duration of spraying the

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
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virus suspension depended on the final RH level required in the experiment and generally ranged between 1 min (low RH) to 10 min (high RH) .

Depending on the experiment, the temperature of the air in the drum cabinet was maintained at either 20±1°C or 6±1°C.

Determination of the Physical Decay: To determine the physical loss of the virus during the processes of aerosolization as well as during the aging of the aerosols, uranine (sodium fluorescein, Fisher Scientific, Fair Lawn, NY, U.S.A.) was used as a physical tracer in all of the aerosol experiments. It was added to the spray fluid at a final concentration of 1 mg/mL. Reference solutions for the standardization of the dye were prepared in TPB. An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Springs, MD, U.S.A.) was used for measuring the dye concentrations in the samples. The excitation and emission wavelengths used were 493.5 and 512 nm, respectively.

Antifoam: In order to minimize excessive frothing during generation and recovery of the aerosols, Antifoam A, (Sigma) was added to the spray and collection fluids at a final concentration of 0.001%.

Test Procedure: The RH and air temperature were first adjusted to the desired level, and an equilibration period of several hours was provided before the aerosolization of the virus into the drum. A Collison nebulizer, with 10 mL of the virus-containing spray fluid, was attached to the inlet of the drum and aerosolization was carried out with compressed air at a pressure of 1.8 kg/cm^2 for the required length of time. The amount of the virus-containing fluid sprayed was determined by weighing the Collison nebulizer before and immediately after aerosolization. Samples of spray fluid were withdrawn before and after the aerosolization process to determine the amount of infecious virus sprayed into the drum.

After aerosolization was complete, a 15-min period was allowed for the stabilization of the aerosol and its even distribution in the drum. An all-glass impinger, containing 10 mL of TPB and 0.001% Antifoam A, was used to collect the first air (time zero) sample from the drum at the end of this aerosol stabilization period. The difference between the 'input virus' concentration and that in the first air sample was regarded as the 'initial biological loss' in the infectivity of the aerosolized virus. It is important to distinguish this 'initial biological loss' of virus, from the subsequent biological decay of the virus (Table 5). For each sample, the impinger was operated for 2 min by means of a vacuum pump to withdraw a total of 11.2 L of drum air. Depending on the duration of the experiment, additional samples of the air from the drum were obtained at 2, 4, 8, 24, 48 and 72 h of aerosol age. To avoid excessive dilution of the aerosols with make-up air during sampling, no more than 5 samples were taken over the entire time-course of any given experiment. After each aerosol sample was collected, the impinger fluid was divided into two portions. One portion was used for the quantitation of the dye and the other, after filtration through a 0.22 um filter, was used for virus plaque assay. At least three experiments were carried out for each set of RH and temperature level tested.

The following formula was used to calculate the initial biological loss of virus as well as the pattern of biological decay of the virus:

$$\% \text{ VIRUS SURVIVAL} = \frac{D_o \times V_t}{D_t \times V_o} \times 100$$

where D_o and D_t are the dye concentrations at time 0 and t hours,

TABLE 5

THE PROCESS OF AEROSOLIZATION, EQUILIBRATION AND BIOLOGICAL DECAY OF VIRAL AEROSOLS

Aerosolization	Equilibration	Survival or Biological Decay Process
1-10 min depending on RH level desired	15 min equilibration period settling of larger particles (> 10 um)	0-72 hours
aerosolization virus subject to shearing and mechanical forces	'droplet nuclei' formation depending on RH and T inside the drum	depends on effect of different experimental conditions (RH and T) on virus survival
influence of virus suspending medium	establishment of aerosol in the drum	biological decay possibly due to denaturation of capsid protein and/or viral nucleic acid

Initial Biological Loss

TIME ZERO

PHYSICAL AND BIOLOGICAL LOSS

BIOLOGICAL DECAY OF VIRUS

respectively; similarly V_o and V_t are the virus titers at 0 and t hours respectively (Sattar et al., 1984). It should be noted that when calculating biological decay, time zero is considered to be the end of the 15 min equilibration period in the drum.

The survival of the aerosolized virus under different RH and temperature conditions was calculated and plotted against time using an exponential model: $y = A\, e^{-Bx}$

The coefficients A and B are decay constants and are estimated by least squares curve fitting. The half-life of the aerosolized virus was calculated from the above formula by regression analysis (Kleinbaum and Kupper, 1978; Lawless, 1982).

PROCEDURE FOR SURFACE SURVIVAL EXPERIMENTS

Contamination of the Disks: Each clean and sterile stainless steel disk (1 cm in diameter), placed in a 24-well plastic plate (Costar, Cambridge, MA, U.S.A.), was contaminated with 10 uL of the suspension, containing approximately 10^7 PFU/mL of RV-14. The inoculum was allowed to air dry in the laminar flow hood under ambient RH and temperature conditions for one hour. The amount of infectious virus remaining after this initial drying period was considered as the 'input virus' level (i.e. T=0).

RH and Temperature Adjustment: The plates containing the disks were then transfered to a transparent glass chamber (21x36x55 cm^3) in a room with diurnal fluorescent light cycles. Virus survival was tested at the same three RH levels used in the aerobiological experiments. For the low RH conditions, air entering the chamber was first passed through a Drierite cylinder. To obtain the high RH, the air was first humidified by bubbling it through deionised water contained in a gas-washing bottle.

During this phase of the study, ambient RH generally remained within the mid-range and required no further adjustments. All the surface survival experiments were carried out at room temperature, which ranged between 20 and 21°C. The RH and temperature were continuously monitored by placing a recording hygrothermogram (Cole-Palmer Instrument Co., Chicago, IL, U.S.A.) inside the glass chamber.

The Test Procedure: The procedure to study the surface survival of RV-14 is based on that developed earlier in our laboratory (Sattar et al., 1986). At appropriate time intervals, the disk to be tested was transfered to a glass vial containing 1 mL of sterile TPB as virus eluent. Sonication (Bransonic, Johns Scientific, Toronto, Ont.) was carried out for 10 min for a thorough elution of virus from the disk. The eluate was further mixed by pipetting several times before being transfered to a 1 mL plastic vial for storage at -80°C.

Statistical Analysis: As in the aerobiological studies, virus survival for each set of experimental conditions was plotted against time using an exponential model; however, to facilitate the statistical analysis, a log-linear plot was made using the least squares curve fitting method. From this plot, an inactivation coefficient, (Ki) of $\log_{10}$ reduction in virus titre/hour was obtained for each set of experimental conditions. The student 't' test (two-tailed) was used to compare the effect of RH on virus survival. Standard errors (SE_y) for each set of experimental conditions was determined, using linear regression analysis. Subsequently, the effect of RH on virus survival was determined by the Ki values and standard errors (SE_y) for each set of experimental conditions using the student 't' test. The statistical analysis for the surface survival experiments is outlined in further detail in the Appendix with examples.

RESULTS

EXPERIMENTAL RESULTS

Effect of Cell Line on RV-14 Plaque Titer: To measure accurately the biological survival of any virus, we need a highly sensitive and reproducible plaque assay system for its quantitation. In preliminary experiments, the L-132 cell line was used for the cultivation and quantitation of RV-14. The A-5 HeLa cell line was reported in the literature to be also suitable for the cultivation and assay of rhinoviruses (Conant et al., 1968). Parallel titrations of RV-14 in both A-5 HeLa and L-132 cells were, therefore, performed, and HeLa cells were found to give a ten-fold increase in the plaque titre of the virus when compared with L-132 cells (Table 6).

The development of plaques was much faster in HeLa cells, where plaques were usually countable by 72 h of incubation; whereas by this time, monolayers of L-132 cells required another 24 h of incubation for the development of countable plaques. Consequently, in all further experiments, the A-5 HeLa cell line was used for the cultivation and quantitation of RV-14.

Effect of Uranine and Antifoam A on Virus Infectivity: Before determining the actual biological decay of the virus, it is essential to account for the dilution of the aerosolized virus in the air, as well as the physical loss that can occur either due to sedimentation or adhesion of the aerosol particles inside the drum apparatus. This can be achieved by adding a 'physical tracer' to the virus suspension to be aerosolized. Various aerobiological studies have made use of tracers such as fluorescent dyes, radioisotopes, and viable bacterial spores for the determination of the physical decay of the virus (Spendlove and Fannin, 1982).

The use of radioisotopes incorporated into the proteins or nucleic

acids of virus particles would be ideal, as each virus particle could then serve as its own tracer (Wolfe, 1961; Spendlove and Fannin, 1982). However, the application of radioisotope tracer techniques is expensive and can be particularly hazardous to human health. The use of fluorescent dyes such as uranine is attractive, because it is relatively inexpensive and safe. Morever, this dye has been shown to be as reliable as radioisotopes as a marker for the physical decay of aerosolized viruses (Ijaz, 1985). Uranine has also been used satisfactorily as a tracer in aerosol studies with adeno- (Songer, 1967), polio- (de Jong et al., 1973), corona- (Ijaz et al., 1985a) and rotaviruses (Sattar et al., 1984). Therefore, this dye was chosen as a physical tracer in all of the aerosol experiments with RV-14. It was added to the virus mixture at a final concentration of 1 mg/mL.

A great deal of froth is generated when TPB is used as a suspending medium for the generation and collection of virus aerosols. To suppress this excessive frothing, it was necessary to add Antifoam A (Sigma) to both the spray and collection fluids at a final concentration of 0.001%.

Since uranine and antifoam A were to be present in the virus suspension fluid, it was first essential to determine if these additives could in any way affect the infectivity of RV-14. Therefore, the virus was suspended in either plain TPB or TPB supplemented with the dye and the antifoam. The virus suspensions were held at room temperature for 30 min before being subjected to rhinovirus plaque assay. Since nearly all of the virus PFU added to the two suspending media could be recovered, it was concluded that the presence of the additives in the spray and collection fluids had no deleterious effect on the infectivity of the virus (Table 7).

TABLE 6

THE PLAQUE TITER OF RV-14 IN TWO DIFFERENT CELL LINES

Expt. No.	PFU/mL A-5 HeLa	L-132
1.	1.50×10^8	1.38×10^7
2.	1.26×10^8	1.07×10^7
3.	1.35×10^8	1.80×10^7
Mean	1.37×10^8	1.41×10^7
S.D.	±0.12	±0.36

Note: Two-day old monolayers of the different cell lines were grown in 80 cm^2 flasks. A standard amount of the virus suspension was added to each flask, adsorbed for one hour and then overlaid with the same overlay medium. Incubation was carried out at 33°C for 72 h in the case of A-5 HeLa cells, and 96 hours for L-132.

TABLE 7

EFFECT OF URANINE AND ANTIFOAM A ON THE INFECTVITY OF RHINOVIRUS-14

Suspending medium	Virus PFU/mL x 10^7
Tryptose phosphate broth (TPB)	1.8
TPB with uranine and antifoam	2.4

Note: Uranine and antifoam were added to TPB to give final conc. of 0.1% and 0.001%, respectively. The virus and suspending medium under test were mixed together and the suspension held for 30 min at room temperature. The samples were plaque assayed in A-Hela 5 cells.

The Effect of Nebulization on the Viability of RV-14: During the process of nebulization, an influx of pressurized air creates a partial vacuum in the Collison apparatus, so that the virus to be aerosolized is subjected to strong shearing and mechanical forces which could lead to its inactivation. It was, therefore, considered necessary to find out if RV-14 could withstand such treatment, before conducting any experiments on the airborne survival of the virus. In order to do this, the nebulizer containing the virus in the spray fluid was operated for 10 min and the samples of the spray fluid collected before and after the process of nebulization were plaque assayed. As can be seen from Table 8, the plaque titre of the virus in the spray fluid remained essentially unaffected by the process of nebulization.

Determination of the 'Initial Biological Loss' in Virus Infectivity: Immediately after the process of nebulization, when the virus cloud is exposed to the new environmental conditions (i.e. RH and temperature) inside the drum air, viral loss could take place due to drying. The drop in the virus titre taking place during this period was termed as the 'initial biological loss' (Table 5). The extent of the initial biological loss depends on the nature of the spray fluid as well as the temperature and RH of the aerosol-receiving air (de Jong et al., 1973; Spendlove and Fannin, 1982). Therefore, in order to differentiate these losses from the actual biological decay of RV-14, an equilibration and stabilization period of 15 min was provided and the initial biological loss of virus infectivity during this period under different experimental conditions of RH and temperature was determined. This was accomplished by comparing the titres of the infectious virus recovered in the first air sample at 15 min to the amount of virus actually aerosolized (using the formula provided in the Materials and Methods Section).

TABLE 8

EFFECT OF NEBULIZATION ON THE VIABILITY OF RHINOVIRUS-14

Expt. No.	Virus PFU/mL x 10^8		% PFU Recovered
	Before Nebulization	After Nebulization	
1	1.62	1.44	88
2	1.41	1.59	112
3	1.50	1.77	118
4	1.04	1.05	100
Mean ± SD			104.5 ± 13.3

Note: The virus was added to TPB with 0.1% uranine and 0.001% anti-foam A. The suspension was placed in a 6-jet Collison nebulizer and it was operated for 10 min. at a pressure of 1.8 kg/cm^2. Portions of the suspending medium collected before and after nebulization were plaque assayed in A-5 HeLa cells.

As can be seen from the results summarized in Tables 9 and 10, when the air temperature was maintained at either 20±1°C or 6±1°C, the initial loss of virus infectivity ranged between 93 and >99.9% at the low and medium RH levels. In contrast to this, there was no detectable loss in virus infectivity at these two air temperatures when the RH level was high.

Decay of Airborne RV-14 at Ambient Temperature: Figure 3 shows the pattern of biological decay of RV-14 when it was aerosolized from TPB and the aerosols maintained at 80±5% RH at ambient temperature over a period of 24 h. Virus half-lives were calculated by regression analysis of the survival data. Under this set of conditions, the half-life of the virus was found to be 13.9±1.9 h and about 30% of the infectious virus could be detected in the drum air even after 24 h of aerosol age. As was mentioned earlier, there was a pronounced initial loss in RV-14 infectivity when the air temperature was kept at 20±1°C and the RH was maintained at either the medium or the low level. Due to this, no estimates of the half-life of the virus under these environmental conditions could be obtained.

In the second series of aerosol experiments at high RH and ambient temperature, the period of observation was extended to 72 h. The half-life of the virus in this case was found to be 13.87±2.05 h. Under these conditions, 15% of the infectious virus coud be detected at 72 h of aerosol age (Figure 4). The estimation of the half-life in this case was found to be almost identical to the results obtained in the first series of experiments at high RH and ambient temperature where the observation period was limited to only 24 h. This confirmed the validity of our mathematical and statistical analysis, where virus survival at 72 h could

TABLE 9

INITIAL BIOLOGICAL LOSS OF RHINOVIRUS-14 DURING AEROSOL STABILIZATION AND EQUILIBRATION AT THREE DIFFERENT RELATIVE HUMIDITIES (20±1°C)

Relative Humidity (%)	Virus PFU/mL		% Initial Loss
	Post spray (Nebulizer)	0.25 hours (Impinger)	
High (80±5)	$2.10\pm0.88 \times 10^{8}$	$1.71\pm1.01 \times 10^{5}$	0
Medium (50±5)	$1.54\pm0.57 \times 10^{8}$	$3.80\pm0.78 \times 10^{2}$	98.78±0.76
Low (30±5)	$1.33\pm0.12 \times 10^{8}$	$1.65\pm0.21 \times 10^{1}$	99.99

Note: Initial biological loss represents the drop in virus titre at the end of the 15 min aerosol stabilization period inside the drum. It is calculated by using the formula provided in the materials and methods section.

TABLE 10

INITIAL BIOLOGICAL LOSS OF RHINOVIRUS 14 DURING AEROSOL STABILIZATION AND EQUILIBRATION AT THREE DIFFERENT RELATIVE HUMIDITIES (6±1°C)

Relative Humidity (%)	Virus PFU/mL		% Initial Loss
	Post spray (Nebulizer)	0.25 hours (Impinger)	
High (80±5)	$1.45\pm0.42 \times 10^{8}$	$6.08\pm1.23 \times 10^{4}$	0
Medium (50±5)	$1.99\pm0.45 \times 10^{8}$	$4.34\pm2.79 \times 10^{3}$	92.9±4.4%
Low (30±5)	$1.20\pm0.38 \times 10^{8}$	$3.30\pm1.27 \times 10^{2}$	98.9±1.40

Note: See Table 9.

Figure 3. The survival pattern of aerosolized RV-14 when tested under high RH conditions (80±5%) and ambient temperature (20±1°C) for a period of 24 hours.

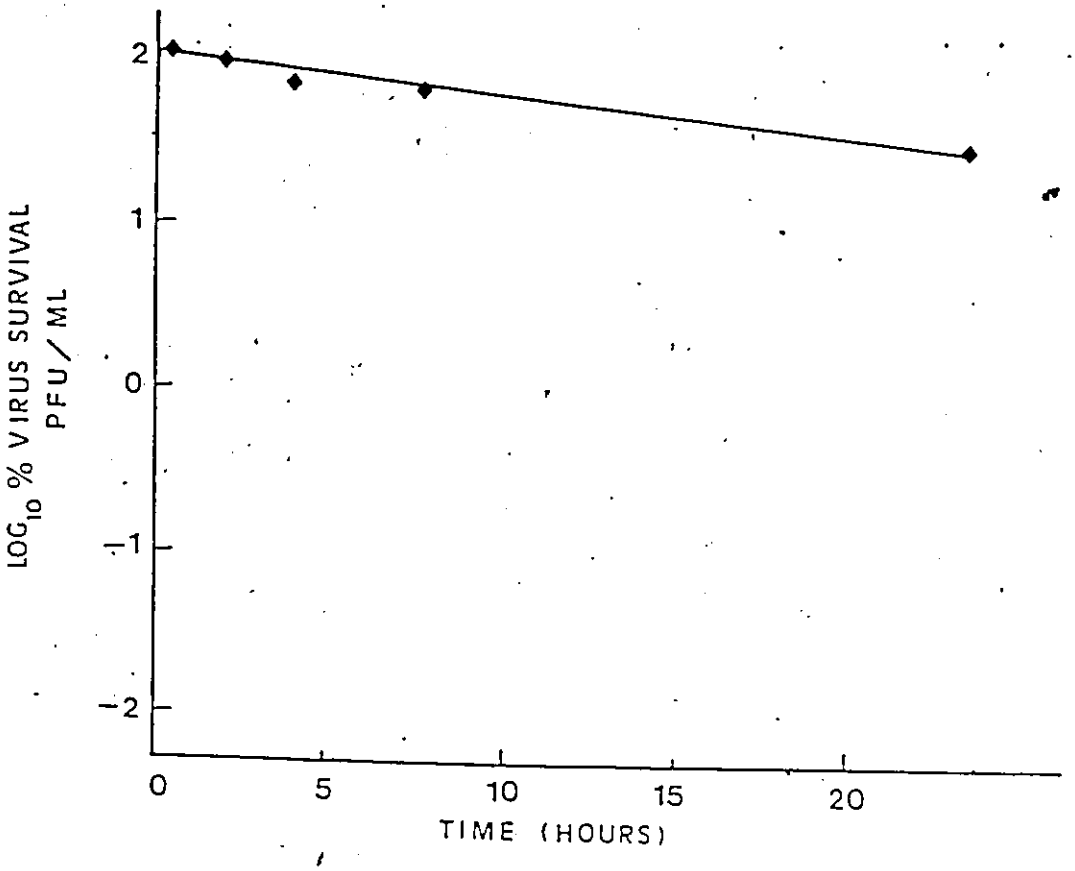
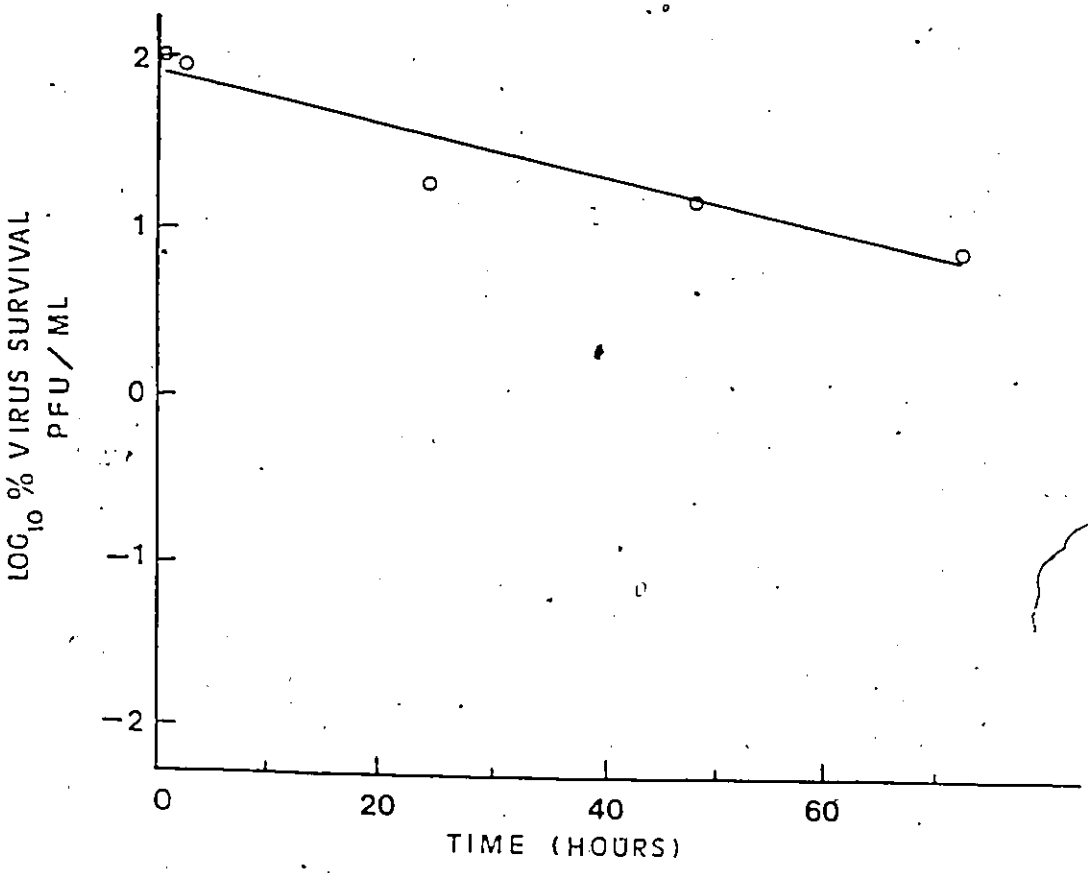

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 4. The survival pattern of aerosolized RV-14 when tested under high RH (80±5%) and ambient temperature (20±1°C) for a period of 72 hours.


2
1
0
−1
−2
LOG10 % VIRUS SURVIVAL
PFU / ML
0
20
40
60
TIME (HOURS)

have been extrapolated just as readily from the 24-hour decay curve without actually doing the extended survival experiment. Consequently, when performing the low temperature experiments, extended survival studies were not done, and virus survival was only tested for up to a period of 24 h.

Decay of Airborne RV-14 at Low Temperature (6±1°C): At the high RH, the half-life was markedly enhanced from 13.9± 1.91 at 20±1°C to 38.9± 4.01 h when the temperature was shifted down to 6±1°C. At this low temperature, over 65% of the airborne infectious virus was still recoverable at the aerosol age of 24 h (Figure 5).

In contrast to the results obtained under conditions of medium RH and ambient temperature, where the virus was rapidly inactivated within the 15 min aerosol stabilization period and therefore no decay curve could be obtained, there was a marked improvement in virus survival at this RH level when the temperature was shifted down to 6±1°C. Figure 6 shows the biological decay pattern of RV-14 at medium RH and low temperature. The half-life was calculated to be 4.09 ±2.05 h, where about 10% of the infectious airborne virus could still be recovered at 24 h.

As can be seen from Table 10, when the air temperature and the RH were kept at the low level, the initial loss of RV-14 amounted to >98% , so that it was not possible to obtain a decay curve or to make any statistical calculations. In this case, the enhancing properties of the low temperature could not offset the deleterious effect of low RH upon airborne RV-14 survival.

Figure 5. The survival pattern of aerosolized RV-14 when tested under high RH conditions (80±5%) and low temperature (6±1°C) for a period of 24 hours.

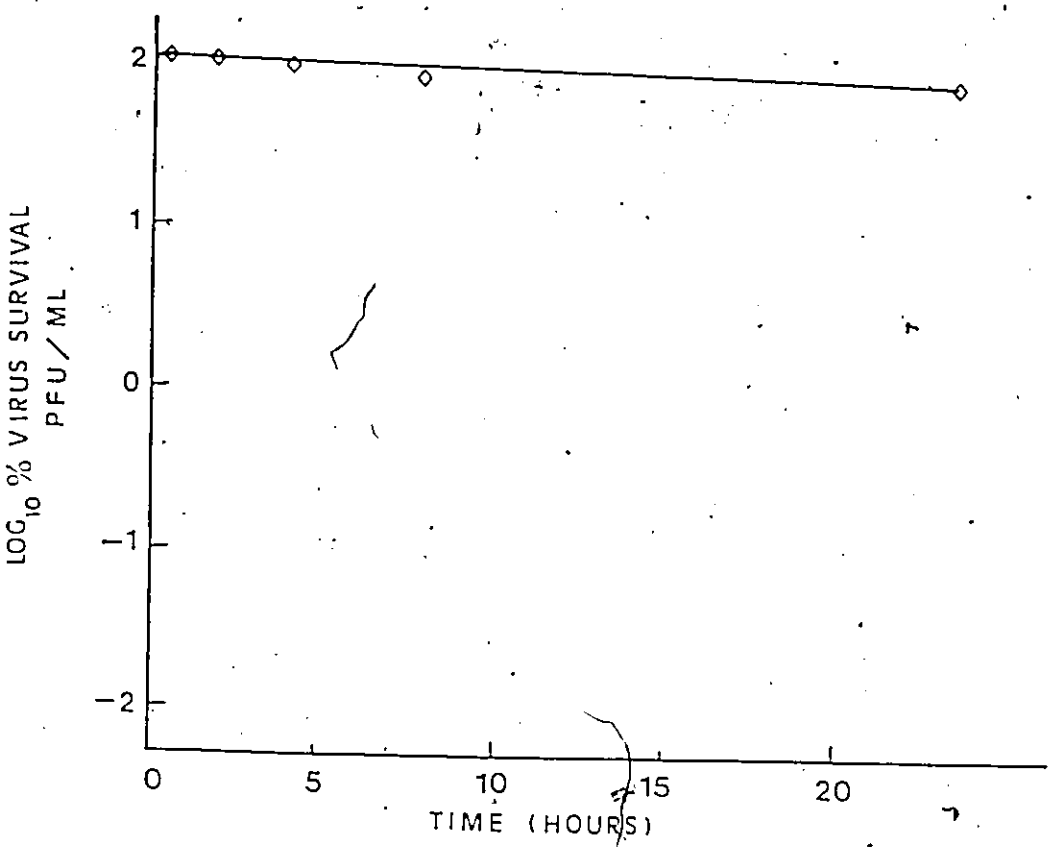
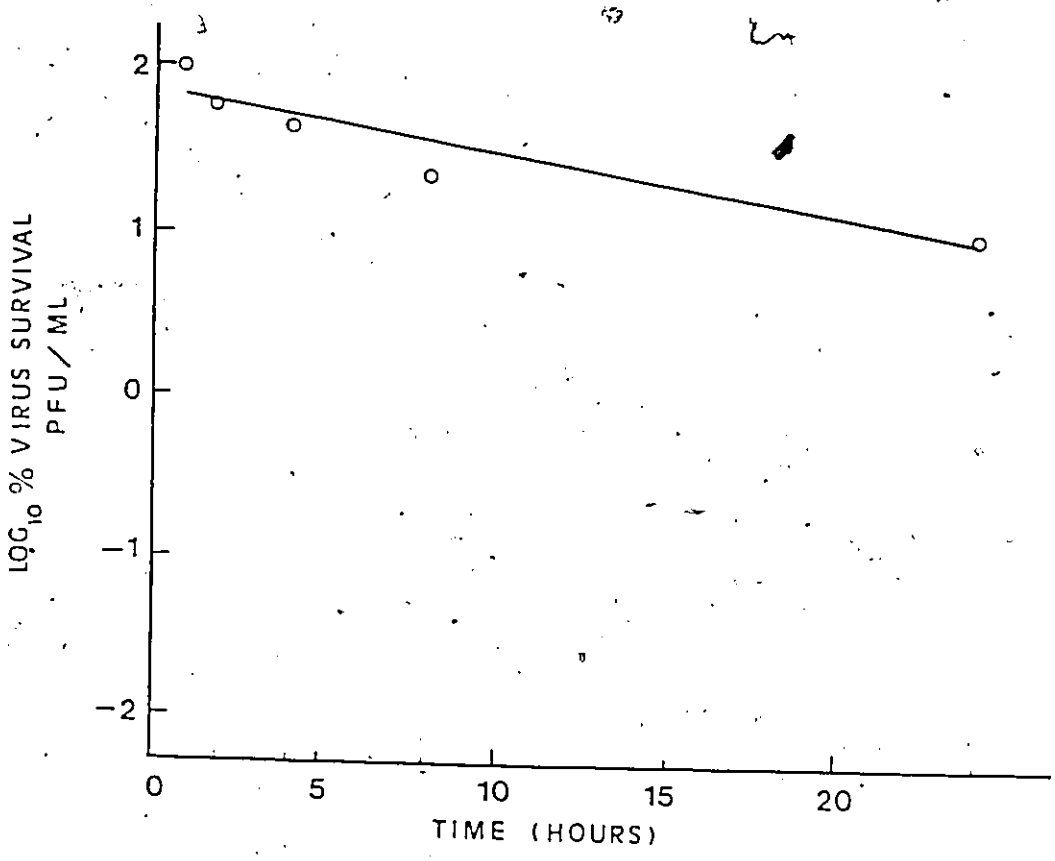

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 6. The survival pattern of aerosolized RV-14 when tested under medium RH conditions (50±5%) and low temperature (6±1°C) for a period of 24 hours.


2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

SURFACE SURVIVAL EXPERIMENTS

Effect of Mucin and Nasal Secretions on RV-14 Infectivity: When the virus was suspended at a 1:10 dilution in either mucin or nasal secretions, and then plaque assayed on A-5 HeLa cells, there was a 40-60% drop in its infectivity (Table 11).

Virus Recovery from Stainless Steel Disks using TPB as Eluent: Using ^{14}C-labelled RV-14 suspended in mucin, the capacity of TPB to elute the virus from the disks was tested. TPB could consistently recover 89-98% of the labelled-virus added to the disks (Table 12). The choice of TPB as eluent is consistent with the observation that proteinaceous substances, polypeptides, and amino acids interfere with the mechanism of virus adsorption to surfaces, and can also be used successfuly to elute previously adsorbed viruses (Gerba, 1984).

The Effect of Drying on Virus Survival on Stainless Steel Disks: Before determining the biological decay of the virus at the three different RH levels, it was important to know the effect of drying on virus survival. After the initial one hour drying period under ambient RH and temperature, virus infectivity was assayed as described. The results for the three suspending media are summarized in Table 13. As can be seen, RV-14 seems to be very susceptible to the effects of drying when suspended in either mucin or nasal secretions. Using mucin as a suspending medium, there was a mean drop of 82% in virus titres at the end of the drying process. A similar pattern was observed when nasal secretions were used as a suspending medium, where there was a mean drop of 89% due to the process of drying. However, when the virus was suspended in TPB, there was only a mean loss of 3% in virus titre due to drying. There seems to be a highly protective effect of TPB upon virus survival during the process of drying.

TABLE 11

EFFECT OF MUCIN AND NASAL SECRETIONS UPON VIRUS INFECTIVITY

Expt. No.	Suspending Medium (Virus PFU/mL x 10^7)		
	MEM Control	Nasal Secretions	Mucin
1.	8.94	3.60 (59.7)	5.34 (40.3)
2.	9.50	2.90 (69.5)	5.20 (45.3)
3.	11.0	4.00 (63.6)	6.11 (44.5)
Mean % Reduction in Virus Titre ± SD		64.2± 4.8	43.3± 2.7

Note: The virus was added to the suspending medium at a dilution of 1:10 and held for thirty min. at room temperature before being plaque assayed on A-5 HeLa cells. The numbers in ( ) represent the percent reduction in virus titre.

TABLE 12

USE OF ^{14}C-LABELLED RHINOVIRUS AS A TRACER IN SURFACE SURVIVAL EXPERIMENTS WITH MUCIN AS SUSPENDING MEDIUM

Expt No.	Time	C.P.M.	% Recovery Radioactivity	Virus Titration PFU/mL A-5 HeLa
1	T_o	966	89	3.0×10^4
2	T_o	1052	97	1.6×10^4
3	T_o	1059	98	2.3×10^4
Control		1083	100	4.5×10^5
	Mean % Recovery ±S.D.		94±5	

Note: 20 ul of the radioactive virus was added to each disk, virus was eluted using 1mL of TPB as eluent. T_o was considered the end of the drying period.

TABLE 13

EFFECT OF DRYING ON RHINOVIRUS-14 SURVIVAL ON STAINLESS STEEL DISKS

Suspending Medium	Mean Viral Loss due to drying (% ±S.D.)	Log_{10} Reduction Titer
Nasal Secretions	89.0±3.20	≥ 1
Mucin	82.0±6.65	≅ 1
TPB	3.0±1.0	0

Note: 10 ul of the virus suspension under test was placed on stainless steel disks and allowed to air dry for a period of one hour in a laminar flow hood under ambient RH and T conditions. A total of three experiments were performed for each suspending medium.

Survival of RV-14 on Stainless Steel Disks at Ambient Temperature under Three Different RH Conditions using TPB as Suspending Medium: In order to better compare the survival patterns of RV-14 in the airborne state to that on surfaces, TPB was used as a suspending medium. Virus inactivation rates (Ki) are summarized in Table 14 for the three levels of RH tested. The decay curves for these experiments are presented in Figures 7-9.

The virus was found to survive best at the high RH level (Ki= .0469); where its inactivation rate was found to be lower than either at the low (Ki= .0686) or medium (Ki=.083) RH levels. This difference was found to be significant at the 95% level of confidence ($P < 0.05$). At the high RH level, there was a less than 0.5 $\log_{10}$ reduction in virus titre at 24 hours. RV-14 survived equally well at the low (Ki= .0686) and medium (Ki= .083) RH levels, where approximately a one $\log_{10}$ reduction in titre of virus occurred after 24 h.

Survival of RV-14 on Stainless Steel Disks at Ambient Temperature under Three Different RH Conditions using Mucin and Nasal Secretions as Suspending Media: When mucin was used as a suspending medium, the virus was found to survive equally well at the low (Ki= .1141), medium (Ki= .1131) and high (Ki= .0851) RH levels (Table 14). Although the inactivation rate at the high RH level (where a <1 $\log_{10}$ reduction in virus titre could be observed after 24 h) was considerably less than inactivation rates at either the low and medium (where a > 1 $\log_{10}$ reduction in virus titre occurred after 24 h) RH levels; this difference was not found to be significant at the 95% confidence level. Figures 10-12 represent the decay curves of the virus for this series of experiments.

TABLE 14

INACTIVATION RATES (Ki) OF RV-14 ON STAINLESS STEEL AT THREEE DIFFERENT RELATIVE HUMIDITY LEVELS

Suspending Medium	(%) Relative humidity		
	20±5	50±5	80±5
Mucin	0.1141	0.1139	0.0851
TPB	0.0686	0.0830	0.0469

Note: Ki is expressed as loss of virus infectivity in $\log_{10}$ PFU/ hour.

Figure 7. The survival pattern of RV-14 on stainless steel disks using TPB as a suspending medium under low RH (20±5%) conditions and ambient temperature (20±1°C).

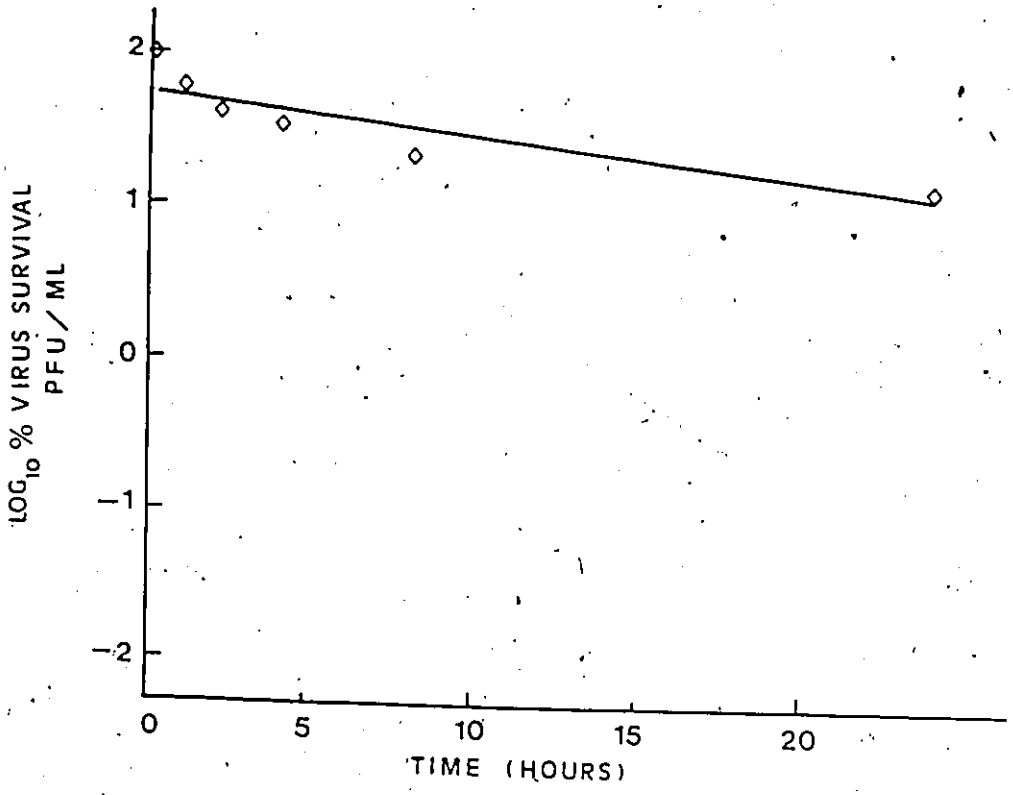

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 8. The survival pattern of RV-14 on stainless steel disks using TPB as a suspending medium under medium RH (50±5%) conditions and ambient temperature (20±1°C).

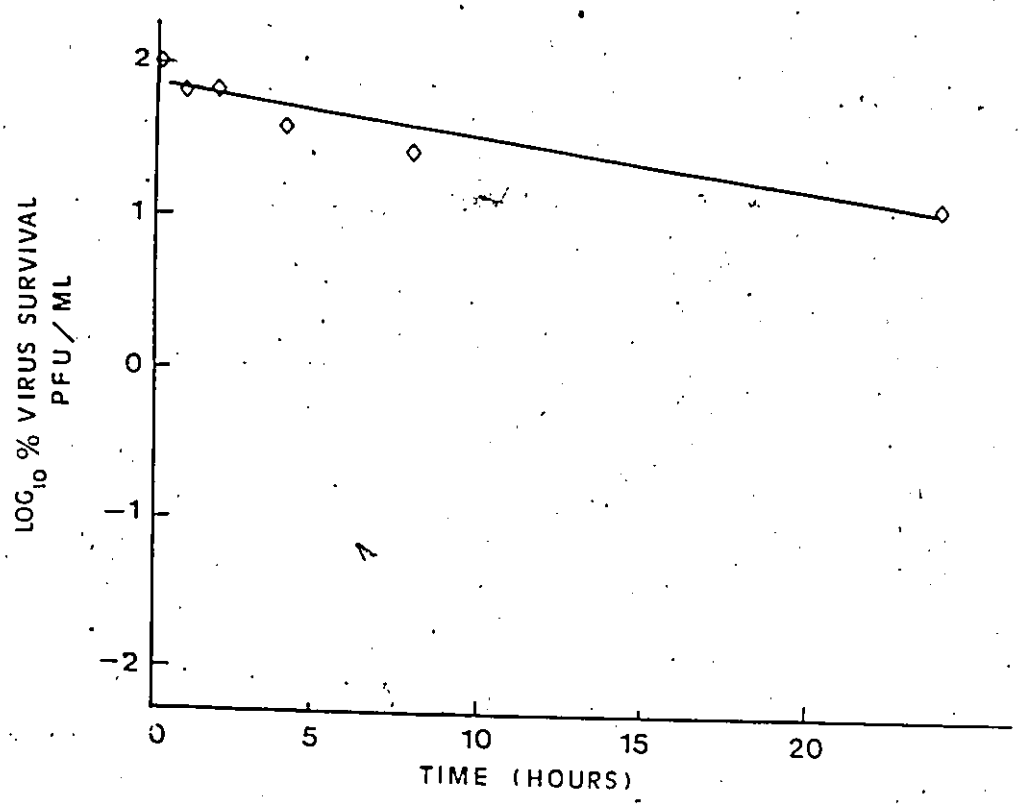

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU/ML
0
5
10
15
20
TIME (HOURS)

Figure 9. The survival pattern of RV-14 on stainless steel disks using TPB as a suspending medium under high RH (80±5%) conditions and ambient temperature (20±1°C).

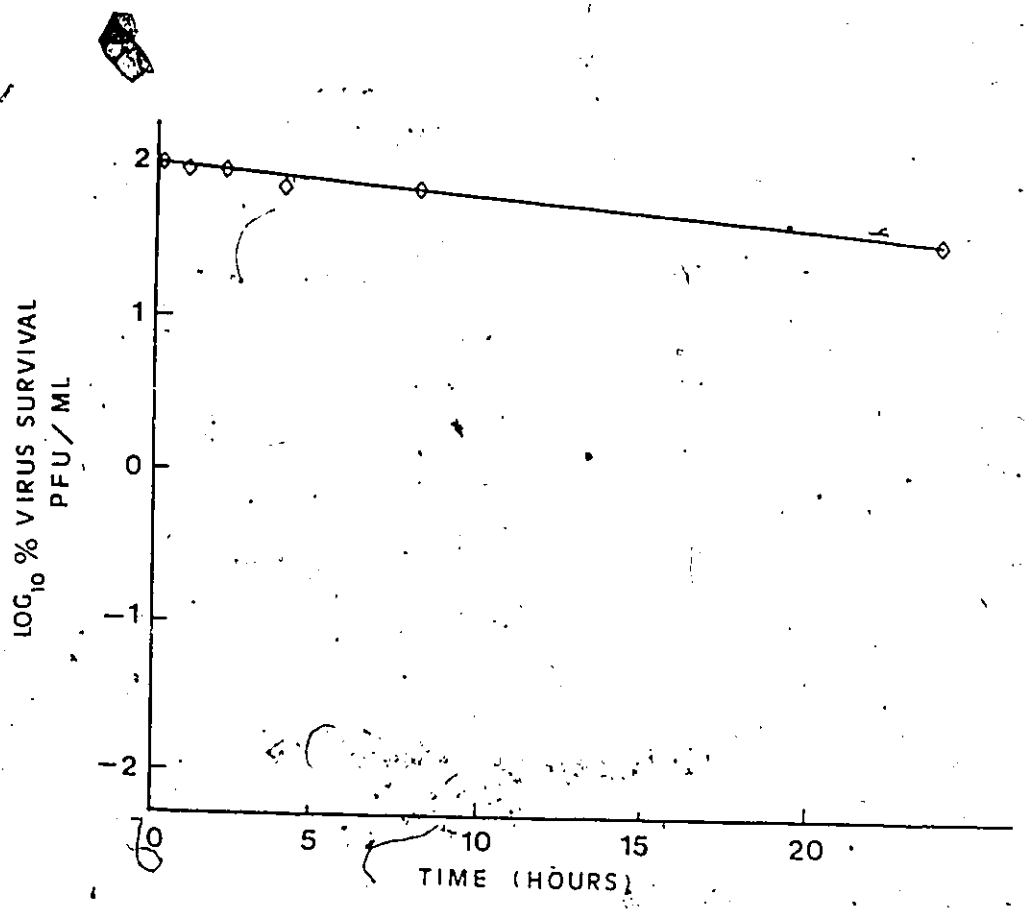

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 10. The survival pattern of RV-14 on stainless steel disks using mucin as a suspending medium under low RH (20±5%) conditions and ambient temperature (20±1°C).

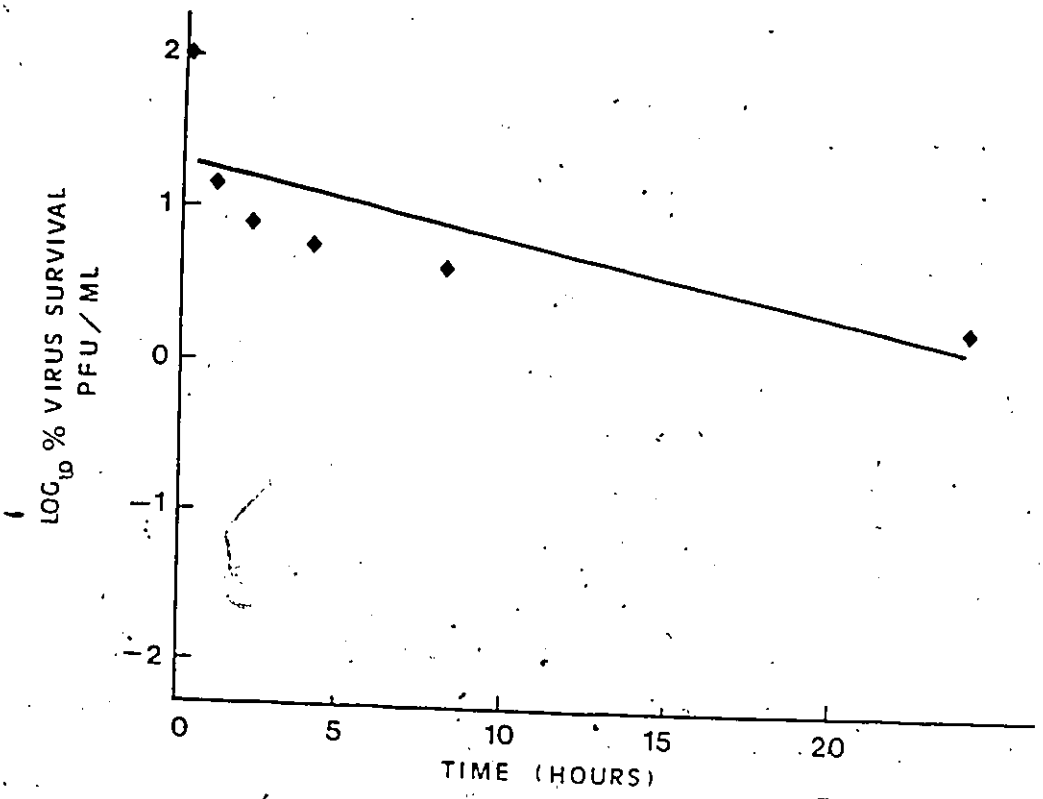

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 11. The survival pattern of RV-14 on stainless steel disks using mucin as a suspending medium under medium RH (50±5%) conditions and ambient temperature (20±1°C).

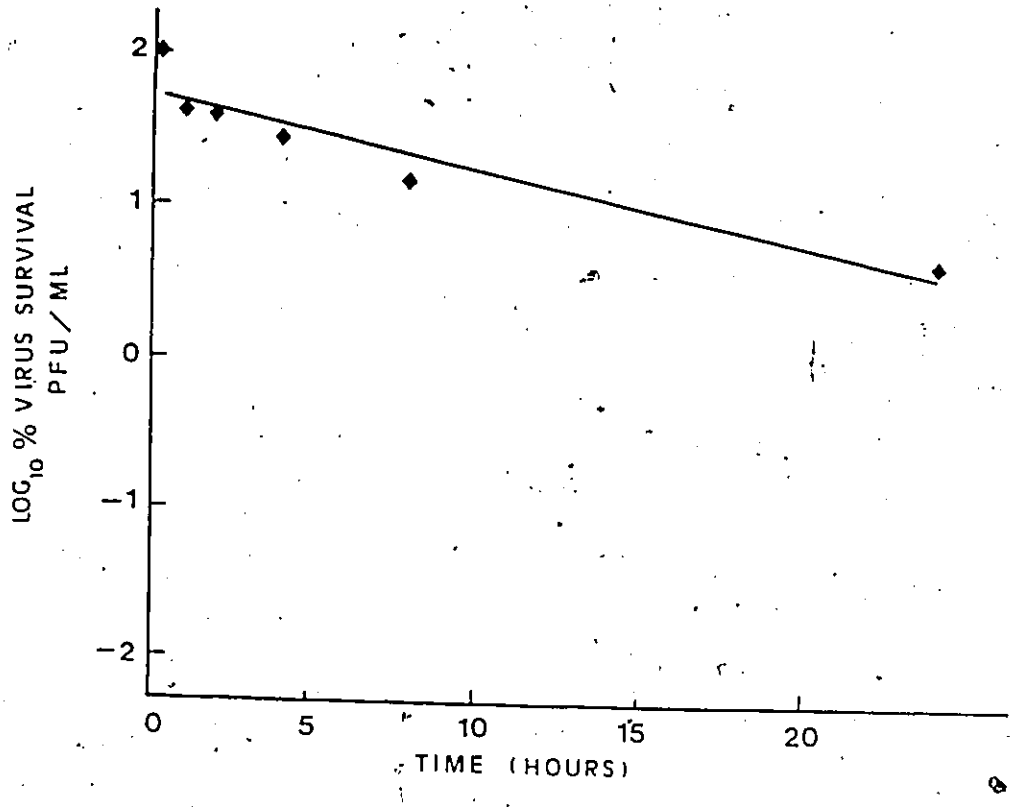
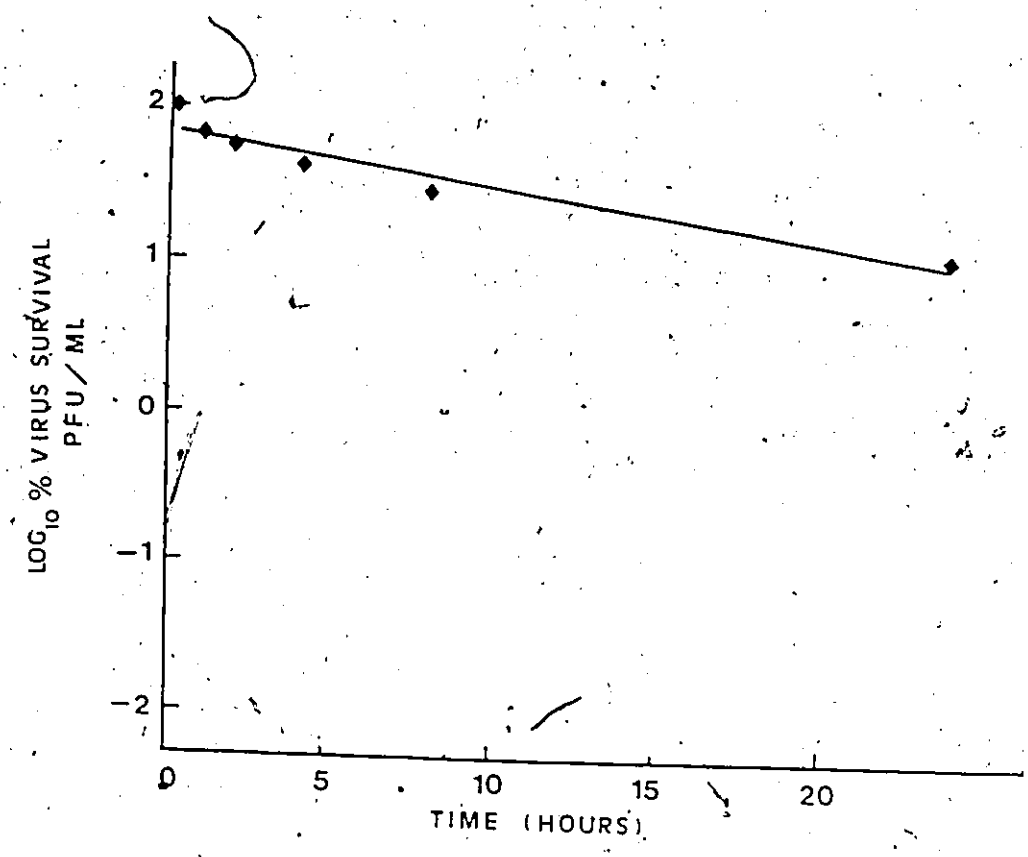

2
1
0
−1
−2
LOG_{10} % VIRUS SURVIVAL
PFU / ML
0
5
10
15
20
TIME (HOURS)

Figure 12. The survival pattern of RV-14 on stainless steel disks using mucin as a suspending medium under high RH (80±5%) conditions and ambient temperature (20±1°C).


2
1
0
−1
−2
0
5
10
15
20
TIME (HOURS)
LOG10 % VIRUS SURVIVAL
PFU / ML

Six experiments were done using nasal secretions as a suspending medium. At the low RH level, the maximum period of virus survival was found to be 4 h. At the high and medium RH levels, it was still possible to recover residual virus up to 8 hours. The results have been expressed as a percentage drop in virus titre for individual experiments as represented in Figures 13-15.

Figure 13. The survival pattern of RV-14 on stainless steel disks using nasal secretions as a suspending medium under low RH (20±5%) conditions and ambient temperature (20±1°C).

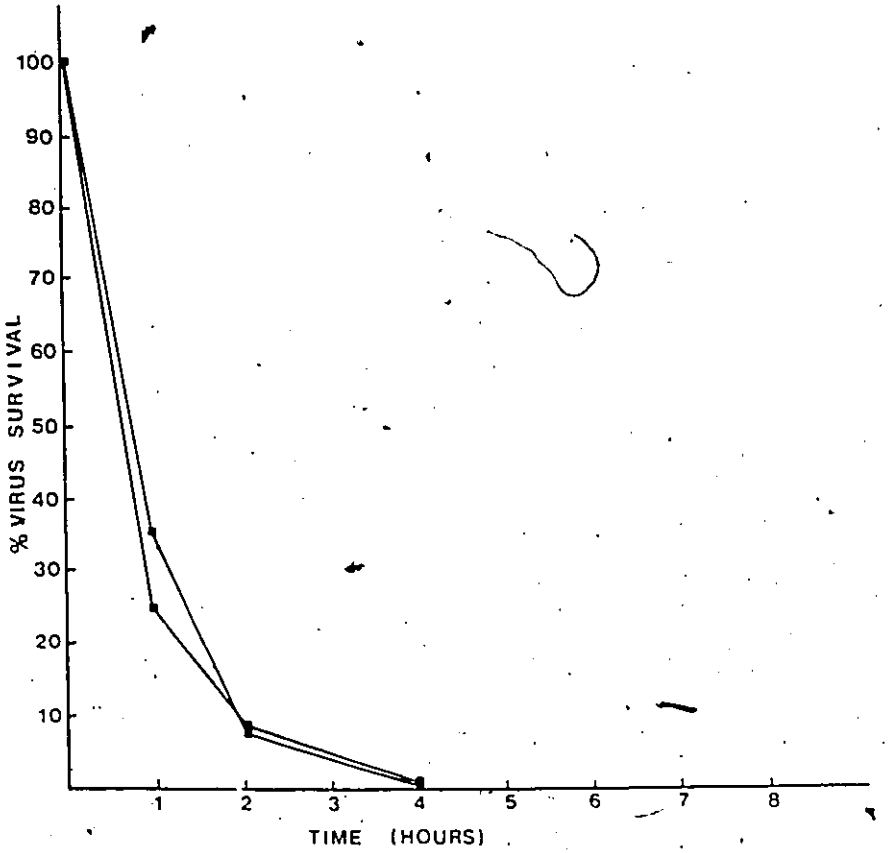

100
90
80
70
60
50
40
30
20
10
% VIRUS SURVIVAL
1
2
3
4
5
6
7
8
TIME (HOURS)

Figure 14. The survival pattern of RV-14 on stainless steel disks using nasal secretions as a suspending medium under medium RH (50±5%) conditions and ambient temperature (20±1°C).

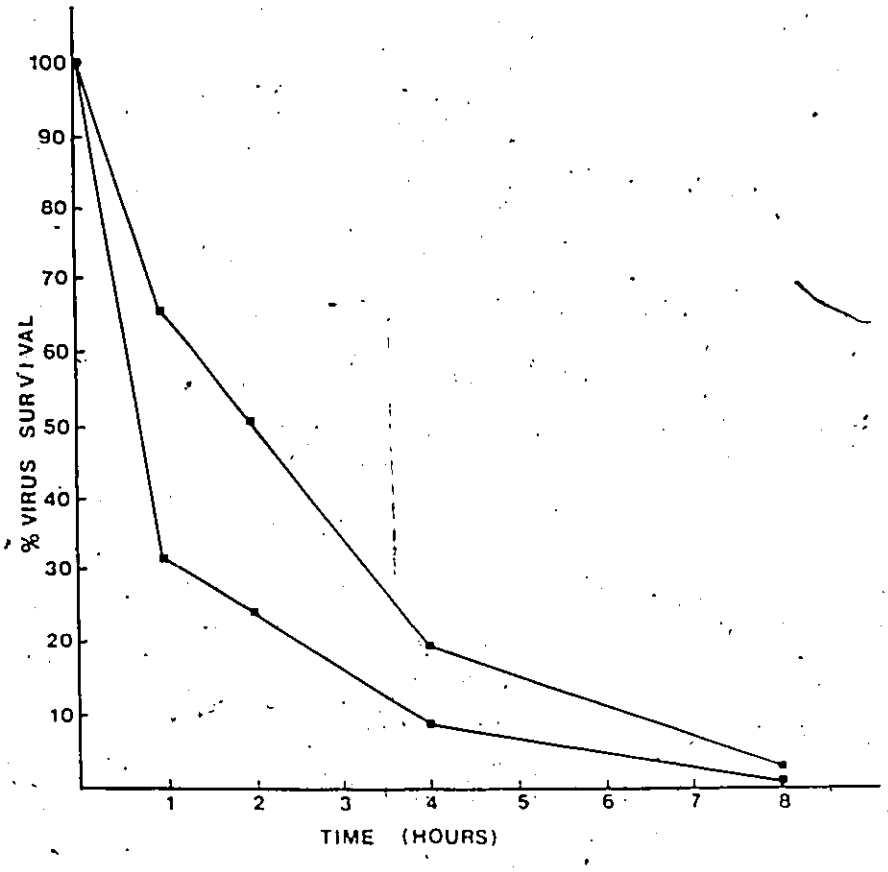
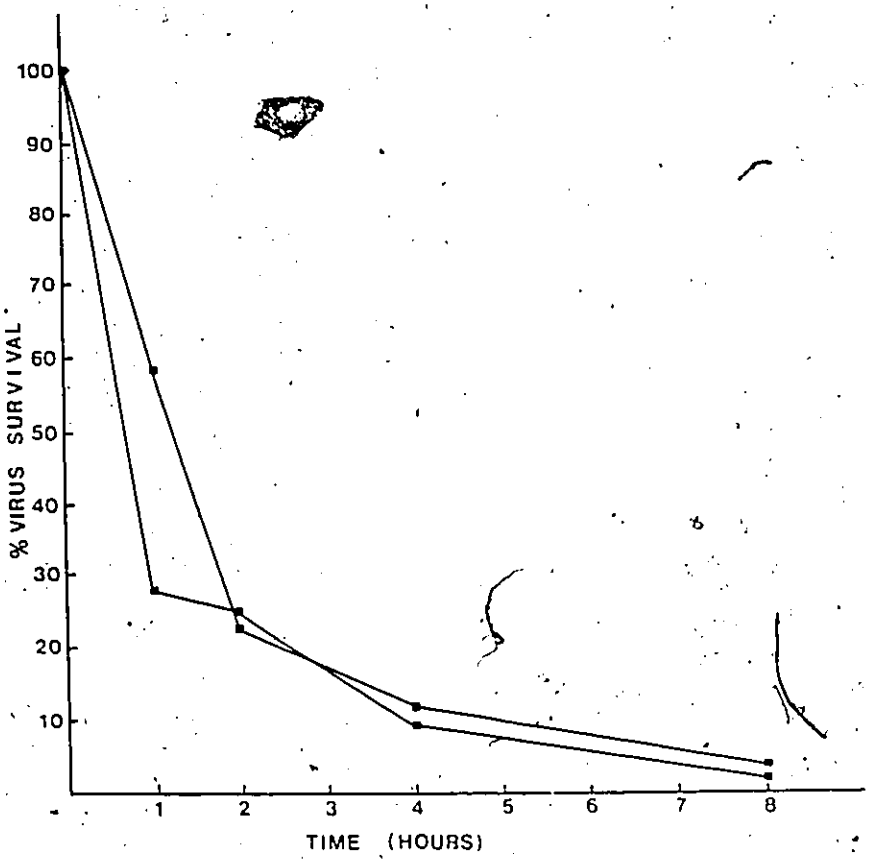

% VIRUS SURVIVAL
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
TIME (HOURS)

Figure 15. The survival pattern of RV-14 on stainless steel disks using nasal secretions as a suspending medium under high RH (80±5%) conditions and ambient temperature (20±1°C).


100
90
80
70
60
50
40
30
20
10
% VIRUS SURVIVAL
1
2
3
4
5
6
7
8
TIME (HOURS)

DISCUSSION

DISCUSSION

Since their discovery, investigators have always been fascinated by the high prevalence of rhinovirus respiratory illness rates and the possible routes of transmission of this large virus group. We now know that there are a number of biological factors which may contribute to the high prevalence of rhinovirus illness in the general population. Some of these factors have been discussed in detail and include:

(1) Emergence of new serotypes, which may be due to recombination or mutation.

(2) Multiple circulating serotypes present simultaneously in the community.

(3) Prolonged periods of virus shedding post-infection.

(4) Low minimal infective dose (MID) required to initiate infection.

(5) Transient protective immunity following infection..

(6) Prolonged rhinovirus survival in the environment.

The aim of this study was to investigate the capacity of rhinoviruses to survive in the environment. Although much work has been done on the survival of rhinoviruses on environmental surfaces, there have been no published studies on the survival of these viruses in the airborne state. The only available report comes from a preliminary abstract on this subject (Holmes et al., 1976a). Their findings suggested that rhinoviruses survived best under high RH conditions. As far as we are aware, further details of this study were never publsihed. In view of this, this investigation was initiated with particular interest in determining which environmental factors promote or retard rhinovirus survival in the airbrone state.

The high and low levels of RH used in this study corresponded to the two extremes in indoor atmospheric conditions generally encountered in

the temperate regions in the summer and winter seasons, respectively. The medium RH was chosen to represent the inside atmosphere of climatically-controlled buildings. The air temperature of 20±1°C was selected to correspond to indoor room temperatures in temperate climates. The low temperature of 6±1°C was selected to represent the cold weather condition such as would be encountered in the fall or winter months. However, because of technical constraints, this was the lowest possible temperature under which survival experiments could be conducted.

Certain criteria must be met, before airborne transmission in viral infections can be substantiated. There must be the generation of aerosols from infectious secretions, which could occur in a variety of situations such as speaking, sneezing, coughing or nose-blowing. Re-aerosolization of virus from virus-contaminated surfaces could also occur by human activities such as dusting, sweeping or toilet flushing (Fig. 1). Furthermore, for airborne transmission to take place, the virus must be able to withstand the process of aerosolization and be able to survive in the airborne state. It is now well established that a variety of environmental factors such as RH and temperature exert a great influence upon virus survival in the airborne state (Benbough, 1971; Spendlove and Fannin, 1982).

Certain other respiratory viruses, for example, influenza and coronaviruses are now suspected to be transmitted primarily by the airborne route (Monto, 1982). Most of the evidence for this comes from epidemiological studies which show that these viruses can spread very efficiently and quickly through a population. These viruses have also been shown to survive very well in the airborne state, over a wide range of relative humidities tested, providing further evidence that air is the likely

vehicle for their transmission (Harper, 1961; Schaffer et al., 1976; Ijaz et al., 1985a). From these studies, it was found that corona- and influenza viruses survive best at the mid (50±5%) and low (17%) RH levels, respectively. Low to mid-range RH atmospheric conditions are often encountered in indoor environments such as in homes, jet-liners, schools, offices and hospitals. These conditions could enhance the survival of such viruses and facilitate their dissemination in confined areas (Moser et al., 1979). Other viruses such as measles have also been found to survive well in the mid to low RH conditions and are thought to be spread primarily by the airborne route (de Jong and Winkler, 1964; Riley, 1974).

Studies on the Survival of Airborne Rhinoviruses: The results obtained from this study indicate that the capacity of rhinoviruses to survive in the airborne state is very limited. At the low and medium RH levels and ambient temperature (which would represent the conditions normally encountered in the indoor environment in temperate climates) the virus was found to be rapidly inactivated within 15 min of aerosolization. Only at the high RH level (80±5%) was the virus found to be capable of survival in the airborne state, with a half-life of 13.9±1.9 h. The pattern of survival at the lower air temperature (6±1°C) was basically the same as seen at the 20±1°C, but with some important differences. At the lower air temperature and the high level of RH, its half-life increased almost three-fold to 38.9±4.01 h. Hence this common cold virus prefered 'damp and cold' conditions for its optimal survival in the airborne state. At the medium RH and low temperature, its half-life (4.09±2.05 h) was still considerably less than what was observed at high RH and ambient temperature. The virus did not survive at all under conditions of both low RH and temperature. In this case, it was concluded that the effects of RH

were more detrimental to rhinovirus survival than the protective effects of lower air temperature.

It should be mentioned that attempts were made to study the airborne survival of RV-14 by aerosolizing it from the one sample of undiluted nasal secretion available to us. Although in one attempt infectious virus could be detected up to the aerosol age of 4 hours when the RH was high and the air temperature kept at 20°C., the limited volume of the nasal secretion prevented us from conducting additional experiments. In addition, the high viscosity of the undiluted nasal secretion appeared to interfere with the process of nebulization. Furthermore, it was felt that the results that could have been obtained using diluted nasal secretion would not have given a realistic picture of virus survival aerosolized from naturally infected individuals. Similar difficulties were encountered when mucin was used as a suspending medium.

The pattern of survival of airborne RV-14 was found to resemble closely that of poliovirus type 1 (Sabin), which was tested previously in our laboratory under identical conditions of RH, temperature, and suspending medium. The half-life of the poliovirus under high RH conditions at ambient temperature was found to be 9.02±1.91 h. Furthermore, as with RV-14, the poliovirus was rapidly inactivated at the medium and low RH levels (Ijaz et al., 1985b). In this regard, the behaviour of rhinoviruses in the airborne state seems to resemble other non-enveloped viruses such as FMDV virus (Donaldson, 1972; Gloster, 1983) or adenoviruses (Miller and Artenstein, 1967), which also prefer high RH conditions for their optimal survival.

Airborne Transmission of Rhinoviruses: As has been outlined in detail in the Literature Review, there have been many experimental studies which

have tried to simulate airborne transmission of rhinoviruses under natural conditions. In most cases they have not been successful in demonstrating airborne transmission. However, there was no reference to the environmental conditions under which these transmission experiments were conducted. These findings at the time were unexpected, as earlier studies on experimental colds suggested that transmission was more likely via the airborne route (Tyrrell, 1965; Cate et al., 1965 ; Couch et al., 1966). If results obtained with the survival of RV-14 in the airborne state are indicative of the behaviour of other rhinovirus serotypes, it is possible that under those particular experimental conditions, the virus did not survive long enough for transmission to take place. Our findings indicate that airborne transmission cannot be discounted entirely, and under conditons of high RH, such as on a damp rainy day, the environmental conditions could be right for airborne rhinovirus infection to be initiated. In this regard, seasonal fluctuations in RH and temperature may be an important factor in the particular seasonality pattern associated with rhinovirus infections.

Studies on the Survival of Rhinoviruses on Surfaces: Direct or indirect contact is now believed to be to be the primary mode of transmission of rhinoviruses, and many studies on the survival of these viruses on surfaces have been conducted. Moreover, transmission experiments under simulated conditions have demonstrated the potential of virus-contaminated surfaces in the spread of rhinovirus colds. These studies have been reviewed extensively in the previous sections. It should, however, be noted that in all of the previous surface-survival studies with rhinoviruses, no attempt was made to control the RH conditions under which the studies were conducted. Therefore, in our experimental set-up we tried to control for this variable. To determine if a

correlation exists between the airborne survival of rhinoviruses and its survival on surfaces, the same range of relative humidities were tested as in the aerosol experiments. As can be seen from Table 13, RV-14 is very susceptible to the effects of drying, and when suspended in nasal secretions, there was a mean loss of 89±3.20% in infectivity after one hour of drying under ambient conditions. This finding is in reasonable agreement with that of Reed (1975), who observed a 40-99% loss of infectivity when rhinovirus type 2 from nasal secretions was dried on skin or other surfaces under ambient conditions. When TPB was used as a suspending medium, there was only a mean loss of 3% in virus infectivity due to the process of drying. Reagan et al., (1981) observed a similar protective effect when BSA was used as a suspending medium in measuring the survival of RV-14 on plastic. It could be that the presence of such proteinacious compounds stabilizes the virus against the deleterious effects of drying.

Also the RH conditions under which the drying process takes place seems to be important. When rhinovirus type B633 was allowed to dry on glass slides under low RH (20%) conditions, a 0.9 $\log_{10}$ reduction in titre was observed; whereas when dried under high RH (84%) conditions, there was only a 0.2 $\log_{10}$ reduction in virus titre (Buckland and Tyrrell, 1962). Similar results with RV-14 were obtained from this study where approximately 0.8 -0.9 $\log_{10}$ reduction in virus titres occurred when the virus was suspended in either mucin or nasal secretions and then allowed to dry under ambient conditions of RH and temperature.

The general survival pattern of RV-14 measured over a 24 h period on stainless steel seemed to correlate well with the results of the airborne survival experiments from the first part of the study. The decay pattern

of RV-14 in the airborne state was very similar to that on stainless steel surfaces using TPB as a suspending medium at the high RH of 80±5%. In both cases, approximately 30% of the infectious virus could be recovered at the sampling time of 24 h. However, the pattern of survival of RV-14 on stainless steel surfaces at the low and medium RH was dramatically different from what was observed in aerosols under the same conditions. RV-14 survived quite well at both of these RH levels on stainless steel for periods upto 24 h (Figures 8-9), whereas in the airborne state it was rapidly inactivated within 15 minutes of aerosol age.

When RV-14 was suspended in mucin and its survival tested over a period of 24 h, again a similar pattern of decay was observed. The lowest decay rate for the virus was observed at the high RH (Table 14), where there was an approximately one $\log_{10}$ decrease in virus titre at 24 h. The difference, however in the decay rates between the low, medium and high RH was not significant ($P>0.05$). Perhaps if a greater number of experiments were to be performed, a significant difference might be observed. The results obtained with mucin as a suspending medium, are not inconsistent with other survival studies on rhinoviruses. A similar reduction in virus titre was observed (1.3 $\log_{10}$) when RV-14 was suspended in Tris-saline-BSA and tested over a period of 24 h under ambient conditions of RH and temperature (Reagan et al., 1981).

When nasal secretion was used as a suspending medium, to our surprise, the virus could be recovered only for 8 hours under high and medium RH conditions and a maximum of 4 hours under low RH conditions (Figures 12-14) This time period is considerably lower than when either TPB or mucin were used as suspending media, where virus recovery was possible at 24 hours. Few investigators have used undiluted nasal secretions as a

suspending medium in investigating rhinovirus survival in the environment. It is interesting to note that Hendley et al., (1973) observed a maximum survival of 5 hours when RV-39 was suspended in undiluted mucus, but when the mucus was diluted 1:3 in physiological saline, virus survival was extended up to 24 hours. However, they did not give any possible explanations as to why this might have occurred. It is possible that the presence of some inhibitory substances in the nasal mucus is inactivating the virus or blocking its infectivity.

CONCLUDING REMARKS

CONCLUSION AND REMARKS

When this study was initiated two years ago, little was known about the survival of rhinoviruses in the airborne state. This study helps to elucidate the role of airborne rhinovirus transmission, and may explain some of the discrepancies in the literature on this subject. In particular, the results obtained both in the airborne as well as surface survival experiments point to the importance of environmental factors (i.e. RH and temperature) on rhinovirus survival.

In summary, the results from all of the survival studies done on this virus indicate that it is highly susceptible to the effects of low RH and dehydration. This effect can be observed in the biological decay of the virus on stainless steel, but is particularly pronounced when the virus is aerosolized. These results also provide further evidence for direct or indirect contact as a primary mode of transmission for rhinoviruses. At ambient temperature, RV-14 survived well on stainless steel at a wide range of relative humidities tested, whereas its capacity to survive in the airborne state was limited to only high RH atmospheric conditions. Lowering the air temperature had a further enhancing effect upon the survival of the airborne virus. However, changes in relative humidity had a more pronounced effect on virus survival than did changes in temperature. The potential for aerial spread of rhinoviruses should be considered particularly under humid and cold conditions. TPB seems to have a stabilizing effect upon virus survival, possibly protecting the virus against dehydration. Suggestions for future investigations include:

(1) Studies on the airborne survival of other serotypes of rhinoviruses could be performed and their survival patterns compared to that of RV-14.

(2) The airborne survival of rhinoviruses in mucus secretions should be tested.

(3) Airborne transmission experiments with rhinoviruses should be conducted with particular reference to the precise environmental conditions of RH and temperature.

(4) Attempts should be made to recover rhinoviruses from artificially and naturally contaminated air using a large volume air sampler.

(5) The survival of rhinoviruses on surfaces held at the lower air temperature could be performed so as to simulate virus survival in the colder outdoor environment.

APPENDIX

APPENDIX

Regression analysis was used as a statistical method for determining the effect of RH on virus survival. This method involves the fitting of a straight line or curve to a scatter of experimentally observed data points.

For each experimental condition, percent virus survival $\log_{10}$ PFU/mL (the dependant variable) was plotted against time (the independant variable) using the least squares curve fitting method. The mathematically-derived line obtained by this method is such that the sum of the squares of deviations of the observed points about the line is a minimum. The slope of the line (B) and the correlation coefficient (R) can be readily obtained from this line.

In order to make inference from this least squares regression line, it is necessary to determine the standard error of the slope of this line (SE_B). From the composite data of at least three experiments, the mean ($\bar{y}$), variance V(y), mean variance S(y) and standard deviation SD(y), of every time interval for each set of experimental conditions was calculated using the following formulas:

$$\text{Mean} = \bar{y} = \Sigma y/n \qquad n = \text{number of observations}$$

$$\text{Variance} = V(y) = \frac{\Sigma(y - \bar{y})^2}{(n - 1)} \qquad y = \text{the ordinate value at each time interval}$$

$$\text{Standard Deviation} = SD(y) = \sqrt{\frac{\Sigma(y - \bar{y})^2}{(n - 1)}}$$

$$\text{Mean Variance} = S(y) = \frac{V(y)}{n}$$

Once the mean variance S(y) has been obtained, the standard error (SE) of the slope (B) which represents the decay constant (Ki) of the virus can be calculated by using the following formula:

$$SE_{(Bn)} = \frac{S(y)\ (1 - R^2)}{n - 2}$$

where:

$SE_{(Bn)}$ - is the standard error of the slope

S(y) - is the mean variance of y

B - is the slope of the line (Ki)

R - is the correlation coefficient

n - is the total number of observations used to determine the mean variance

The standard error of the B values for each set of experimental conditons (i.e. differing RH levels) can be compared directly in the 't' test (2-tailed) using the following formula:

$$t = \frac{B_1 - B_2}{\sqrt{SE_{(B1)}^2 + SE_{(B2)}^2}}$$

where t is the test statistic with $(n_1 + n_2 - 4)$ degrees of freedom

EXAMPLES:

1) Virus recovery (log_{10} % PFU/mL) from survival studies on stainless-steel disks held at 20± 1°C and medium (50±5%) RH conditions using TPB as suspending medium.

Time (hours)	Experiment No. (log_{10} % PFU/mL) 1.	2.	3.	y	± SD	V(y)
0	2.00	2.00	2.00	-	-	-
1	1.83	1.77	1.81	1.80	0.0301	0.000909
2	1.87	1.76	1.78	1.81	0.0592	0.003503
4	1.44	1.66	1.55	1.55	0.1078	0.011600
8	1.38	1.27	1.51	1.39	0.1214	0.014737
24	1.00	0.96	1.18	1.04	0.1202	0.014480

2) Virus recovery (log_{10} % PFU/mL) from survival studies on stainless steel disks held at 20±1°C and high (80±5%) RH conditions using TPB as a suspending medium

Time (hours)	Experiment No. (log_{10} % PFU/mL) 1.	2.	3.	y	±SD	V(y)
0	2.00	2.00	2.00	-	-	-
1	1.93	2.01	1.91	1.97	0.0922	0.00849
2	1.90	2.00	1.90	1.93	0.0609	0.00371
4	1.83	1.91	1.79	1.84	0.0600	0.00360
8	1.91	1.81	1.78	1.83	0.0734	0.00539
24	1.32	1.34	1.70	1.45	0.2139	0.04580

Summary of statistical analysis for results of survival studies on RV-14 at ambient temperature (20±1°C) under three different RH conditions with TPB as suspending medium.

% Relative Humidity	R	B (Ki)	n	S(y)	SE
20±5	0.872	0.06867	24	0.01860	0.01426
50±5	0.938	0.0830	18	0.00750	0.00760
80±5	0.990	0.0469	18	0.01117	0.00370

Summary of statistical analysis for results of survival studies on RV-14 at ambient temperature (20±1°C) under three different RH conditions with mucin as suspending medium.

% Relative Humidity	R	B (Ki)	n	S(y)	SE
20±5	0.742	0.1141	18	0.24797	0.0834
50±5	0.938	0.1139	18	0.0076	0.0075
80±5	0.957	0.0851	18	0.0313	0.0128

SAMPLE CALCULATION USING DATA GIVEN ABOVE:

The t- test (2-tailed), comparing the survival of RV-14 with TPB as suspending medium at 80±5% (B_1) and 50±5% (B_2) relative humidity levels.

$$t = \frac{B_1 - B_2}{\sqrt{SE_{(B1)}^2 + SE_{(B2)}^2}}$$

$$t = \frac{0.0830-0.0469}{\sqrt{(0.00768)^2 + (0.00370)^2}}$$

$$= \frac{0.0361}{\sqrt{0.0000589 + 0.0000136}}$$

$$= \frac{0.0361}{0.0085205}$$

$$t = 4.24$$

Degrees of freedom
$= n_1 + n_2 - 4$
$= 18 + 18 - 4$
$= 32$

$t_{32}(0.05) = 2.04$

Therefore $P < 0.05$

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