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STUDIES ON THE SURVIVAL AND CHEMICAL DISINFECTION OF HUMAN ROTAVIRUS (Wa) ON INANIMATE SURFACES

A thesis submitted to the
School of Graduate Studies
University of Ottawa

In partial fulfillment of
the requirements for the Degree of
Master of Science,
Department of Microbiology and Immunology,
School of Medicine.

by
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September 1985

ISBN 0-315-30980-6

Dedicated

to

My Family

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ACKNOWLEDGEMENTS

I am very grateful to Dr. S. A. Sattar, my thesis supervisor, for his guidance during the investigations and preparation of this thesis. He was most patient, understanding and encouraging throughout my stay here.

For assistance whenever needed and many helpful discussions, Mrs. V. Susan Springthorpe is gratefully acknowledged. I thank Dr. A. Springthorpe for the computer programme used for data analysis and Dr. R. C. Nair for advice on the statistical analysis of the data.

I would also like to thank all the members of the teaching staff, my fellow graduate students and support staff of the Department of Microbiology and Immunology, for their cooperation always. I thank Jodi Grenier for laboratory support during the early phase of this work and May Tawia Coker for assistance in the typing of this thesis. The companionship of Reina Laura Rivera is much appreciated. The members of my thesis advisory committee, Mrs. M.E. Kennedy, Bureau of Infection control, Health and Welfare Canada, and Mr. L. McClelland, Pesticides Division, Agriculture Canada, were most generous with their time and provided me with useful information, constructive criticisms and advice.

The International Development Research Centre (IDRC), Ottawa, generously provided me with a scholarship to enable me to undertake this study. I am particularly grateful to Dr. Karl Smith, Dr. A. Rix, Mrs. L. Rohoncsy, Dr. C. Smart, Mrs. J.S. Bustos and Mrs. A. Guay for their assistance throughout my dealings with the IDRC.

I am indebted to Dr. F. Oldfield, Director of Medical and Health Services, The Gambia, and Dr. B. M. Greenwood, Director of Medical Research Council Laboratories, Fajara, The Gambia, who were most encouraging and supportive of my desire to undertake graduate studies.

SUMMARY

The potential of fomites and environmental surfaces as vehicles in the transmission of rotaviral diarrhoea was evaluated. Disks (1 cm diameter) of various non-porous (stainless steel, glass, and two types of plastic) and porous materials (cloth, poster card, paper currency and four different types of stationery paper) were contaminated with about 10^5 plaque forming units of the Wa strain of human rotavirus (HRV) grown in MA-104 cells and suspended in faecal matter. The inoculum was allowed to dry for two hours under ambient conditions of temperature and relative humidity (RH) and the contaminated disks were held, unless otherwise indicated, for ten days at 22°C or 4°C with the RH at the high (85±5%), medium (50±5%) or low (25±5%) level.

The pattern of virus survival was the same irrespective of the type of non-porous material tested. When the disks were held at 22°C, there was a <1 $\log_{10}$ reduction in virus infectivity after ten days at medium and low RH; at high RH, virus infectivity became undetectable within two days. At 4°C, the virus survived even better at the mid RH level with essentially no change in virus infectivity titer over the ten day period; there was <1 $\log_{10}$ drop in virus titre over 6 days when the RH level was high.

The survival of HRV on porous materials was variable. On currency paper and poster card, HRV infectivity became undectectable during the initial drying process. In preliminary experiments on the other types of paper, nearly all of the in-put infectious virus could be recovered at the end of 2 hours drying time. On cloth, HRV infectivity remained detectable for 2 days at 22°C and up to 6 days at 4°C when the RH level was at the mid range.

In view of these findings, experiments were carried out to evaluate

the rotavirucidal efficacy of various products commonly used for disinfection and antisepsis. For these experiments, the product under test was applied to HRV-contaminated disks of non-porous materials. After one minute of contact the action of the disinfectant was terminated by dilution in tryptose phosphate broth. A disinfectant was considered effective if it could reduce the virus titre by at least 3 $\log_{10}$.

Only 33.3% (9/27) of the formulations tested proved to be effective by our standards. Further testing of the effective ones showed that all of them were in fact able to reduce the virus titre on the contaminated surfaces by at least 6 $\log_{10}$.

These findings suggest that under certain environmental conditions a variety of HRV-contaminated surfaces could play a role in the transmission of rotaviral gastroenteritis. Furthermore, the relatively high resistance of HRV to many commonly used chemical disinfectants and antiseptics emphasises the need for a more careful screening and selection of such products for use in the control and prevention of rotaviral infections.

LIST OF ABBREVIATIONS

AV 11	adenovirus Type 11
ARG	arginine
BSA	bovine serum albumin
°C	degrees centigrade
CHEO	Children's Hospital of Eastern Ontario
cm	centimeter
cm^2	square centimeter
cm^3	cubic centimeter
CO_2	carbon dioxide
CsCl	cesium chloride
DDW	deionised distilled water
DNA	deoxyribonucleic acid
EBSS	Earle's balanced salt solution
e.g.	for example
et al.	and other (others)
EV-70	enterovirus 70
FCS	foetal calf serum
g	gram
g	gravitational force
GLY	glycine
h	hour
HBV	hepatitis B virus
HRV	human rotavirus
HSV	herpes simplex virus
Ki	inactivation coefficient
L	litre

MEM	Minimal essential medium (Eagle's)
mg	milligram
min	minutes
mL	millilitre
mths	months
NaOH	sodium hydroxide
nm	nanometre
P	probability
PBS	phosphate buffered saline
PFU	plaque forming units
ppm	parts per million
PPV	porcine parvovirus
PRV	pseudorabies virus
RH	relative humidity
RNA	ribonucleic acid
RSV	respiratory syncytial virus
rpm	revolutions per minute
SA-11	simian rotavirus SA-11
TCF	tissue culture fluid
TGV	transmissible gastroenteritis virus
TPB	tryptose phosphate broth
uCi	microcurie
ug	microgram
uL	microlitre
um	micrometre
v/v	volume for volume
w/v	weight for volume
-ve	negative

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GENERAL INTRODUCTION

As infectious agents, viruses contribute enormously to human debility and death. On a global basis, an estimated 200 million persons are believed to be chronically infected with viruses (Kurstak, 1984). Confirmation of a viral aetiology is often difficult but more than 90% of respiratory infections and more than 60% of diarrheal episodes, especially in children, have been estimated to be viral in origin (Evans, 1982). Safe and effective chemotherapeutic agents are not yet available against most viral infections and at the present time only a limited number of viral diseases can be controlled and prevented by vaccination. Empirical use of disinfectants in counteracting the spread of viral diseases has led to equivocal success.

There are also serious gaps in our understanding of how many common viral infections spread in nature. Undoubtedly, an important factor in the epidemiology of many of these diseases must be the ability of the virus to survive outside the body of the host. However, very limited data are available regarding the capacity of many important human pathogenic viruses to survive in nature, nor do we know the extent to which virus survival is influenced by various environmental factors. Such information would be essential for the proper control and prevention of viral infections.

Rotaviruses frequently cause outbreaks of acute gastroenteritis in humans. However, the exact mechanisms of virus transmission in such outbreaks have not yet been clearly established. We also do not fully understand why many outbreaks of rotaviral diarrhoea are seasonal in nature. Previous studies in this laboratory have examined the possible role of drinking water (Raphael, Sattar & Springthorpe, 1985) and indoor air (Ijaz et al., 1985b) as vehicles in the spread of rotaviral infections. The present studies were undertaken to determine if environmental surfaces and fomites could also play a role in the transmission of these viruses.

METHODS FOR VIRUS RECOVERY FROM ENVIRONMENTAL SURFACES AND FOMITES

Several sampling methods are utilised for the recovery of viruses from different types of environmental surfaces and fomites (England, 1982).

The rinse method

This technique is most often employed for objects small enough to be immersed easily into medium contained in another vessel (Borick et al., 1964; Gaustad et al., 1974). The virus is then eluted from the entire surface of the fomes by vigorous agitation and or sonication of the contents of the vessel. The infectious virus eluted into the medium is subsequently quantitated by an appropriate assay system. This method generally gives a high virus recovery because the entire surface is in contact with the eluent. In some instances, this procedure needs relatively large volumes of the eluent. This inevitably leads to dilution of the recovered virus, which requires concentration of the eluate prior to virus quantitation.

The wipe-rinse method

Here, a swab (Favero et al., 1974; Hall et al., 1980; Bond et al., 1983) or a piece of cloth (Petersen et al., 1973) is first soaked in the eluent and rubbed over a known area of the surface to be sampled and then immersed in a measured volume of the eluent. The eluate recovered is assayed for infectious virus. This method has been found to be most effective in situations of very high contamination on a limited surface area.

In a comparative study (Phillips and Grim, 1965), calcium alginate swabs (frequently used for the recovery of bacteria from surfaces) were compared to those made with cotton wool and were found to be equally efficient in the recovery of several types of picornaviruses from experimentally-contaminated surfaces.

Miscellaneous methods

For porous surfaces, the material is generally homogenised or macerated followed by thorough rinsing of the treated material in the relevant collection fluid. The resultant suspension is either filtered or centrifuged and the filtrate or supernatant fluid assayed for infectious virus.

Where a large object or a wide area with a non-porous surface is to be sampled, a small volume (5-10 mL) of the eluent is sometimes used to repeatedly wash a demarcated area (Hall et al., 1980).

FACTORS INFLUENCING VIRUS SURVIVAL ON ENVIRONMENTAL SURFACES

Although viruses are obligate intracellular parasites, many of them are capable of surviving outside the host body for protracted periods (Table 1). The capacity of viruses, having different chemical compositions (including the presence or absence of a lipid envelope), to survive on fomites and environmental surfaces is influenced by a number of factors such as:

1) air temperature
2) relative humidity (RH)
3) nature of the virus suspending medium
4) the type of surface

Air temperature

In general, virus survival in the environment is inversely proportional to air temperature. However, the exact mechanisms by which air temperature affects virus survival on environmental surfaces is not fully understood. Ginoza (1968) suggested that the inactivation of viruses by air temperature is a process which proceeds in a manner characteristic of the chemical components of the virus, namely the protein capsid, the RNA or the DNA core; with the inactivation of each occurring at a different rate relative to the other.

Relative humidity

Viruses are dissimilar in the degree to which they are affected by the RH of their environment. Buckland & Tyrrell (1962) studied a wide range of viruses in order to determine the effect of RH on their capacity to survive on various types of surfaces. They noted that non-lipid containing viruses (eg. enteroviruses & adenovirus) survived best at high RH, while those which have lipid components (eg. influenza & arboviruses) were more stable at low RH. However, the data summarized in Table 1 indicate that there are many exceptions to this rule.

Ward and Ashley (1977) investigated the effect of drying on enteric virus (polio virus, reovirus & coxsackievirus) survival. They demonstrated that infectivity of these viruses was lost during the "dewatering" process of evaporation, and also showed viral particles had broken down and released their RNA during the evaporation process. This was shown by density gradient centrifugation of the samples. An interesting finding was that most of the viral particles were inactivated while in solution and not in the dry state when adhering to the sides of the beakers. They suggest that the mechanism here may be the accelerated exposure of viral particles to air at the liquid-air interface, causing inactivation of the virion by release of its genome. Studies by Trouwborst et al. (1974) on the effect of liquid/air interfaces on virion integrity support this hypothesis.

Nature of the virus suspending medium

The nature of the medium in which virus is suspended before surface contamination occurs has a significant effect on virus survival. In experimental studies, presence of proteinaceous matter and inorganic salts in the suspending fluid has often been found to confer stability on the virus. McGeady, Siak & Crowell (1979) studied the survival of coxsackievirus B3 suspended in different salt solutions and bovine serum albumin (BSA). They

were able to demonstrate that BSA conferrred stability on the virus as did salt, but to a more limited extent and that the effect of both seemed to be additive. Inactivation of the virus occurred most rapidly at 37°C but even at this temperature the protein and salt appeared to exert their stabilising effect on virus infectivity. The presence of protein and organic and inorganic salts protected rhinovirus types 2 and 14 infectivity during the process of drying (Reagan, McGeady & Crowell, 1981). Hendley, Wenzel & Gwaltney (1973), working with rhinovirus 39, observed that the virus survived better after drying in saline or other inorganic salt solutions than it did in the presence of nasopharyngeal secretions.

Studies with herpesviruses (HSV) showed that, when suspended in saliva (Turner et al., 1982) or secretions from genital lesions (Larson & Bryson, 1985), could survive for up to 24 and 72 hours respectively, on hard nonporous surfaces. Nerurkar et al. (1983) however, studying the survival of HSV on plastic suspended in cell culture medium, found that virus infectivity became undetectable after 4.5 hours. Interestingly, virus suspended in distilled water could survive on plastic for at least 24 hours.

In experiments with influenza A virus, Edward (1941) noted that the amount of virus that survived and persisted after drying in the presence of saline or serum-broth was the same, but significantly less virus survived after drying in saliva. He also suggested that this apparent loss of virus infectivity in the presence of saliva may be due to a direct destructive action on the virus by a lysozyme-like agent.

The type of surface

A number of investigators have studied the effect of surface type on virus survival. The findings of Hall, Douglas & Geiman (1980) suggest that the potential for spread of RSV in infant secretions via surfaces depended more on the type of surface contaminated than on the ambient

conditions or drying time of the virus in these secretions. They found that survival of RSV was greater on the less porous surfaces, such as countertops, rubber gloves and glass than on porous surfaces, such as cloth and paper tissues. The poor survival of this virus on human skin was thought to be related to the virucidal effects of substances that may normally be present on skin. Hendley, Wenzel & Gwaltney (1973), working with rhinoviruses of human origin, found that under ambient conditions the viruses survived better on formica, stainless steel and wood when compared to several fabrics, facial tissue and paper towels. They also noted that these viruses remained viable on human hands for several hours.

Studies with polio- and vaccinia viruses have shown that they could survive for several weeks on a variety of fabrics (Sidwell, Dixon & McNeil 1966; Dixon, Sidwell & McNeil 1966). These experiments further revealed that virus survival was better on wool than on cotton materials. Mahl & Sadler (1975) compared the survival of adeno-, entero-, herpes- and vaccinia viruses on a selection of hard inanimate surfaces under different atmospheric conditions; the type of surface as such was not found to make a difference in virus survival.

No difference in the survival capacity of faecally-suspended human rotavirus on glass coverslips and cotton swabs was observed in the study by Moe & Shirley (1982). Likewise, Favero et al. (1974) showed that hepatitis B virus could remain viable on both stainless steel and cotton swabs for up to 14 days. McGeady et al. (1979) also found that there was no basic difference in the capacity of coxsackievirus B3 to survive on glass, plastic and paper.

ENVIRONMENTAL SURFACES AND FOMITES AS VEHICLES IN THE SPREAD OF VIRAL INFECTIONS

The available epidemiological evidence has led to the recognition of

Table 1.

SURVIVAL OF VIRUSES ON SURFACES: DOCUMENTED REPORTS

VIRUS	SOURCE OR SUSPENDING MEDIUM	SURFACE	SURVIVAL DURATION	ENVIRONMENT RH (%)	ENVIRONMENT TEMP (°C)	REFERENCES
Variola	Scabs	dried skin	3-4mths	58	30	MaCullum & McDonald 1957
	"		2-4mths	73 & 84	30	
ECHO 1	Liquid gum	gum dried on paper	30 days	ambient	ambient	Selwyn 1965
Adeno 3	"	"	10 days	"	"	"
Vaccinia	Tissue culture	cotton,	14 weeks	35	25	Sidwell et al. 1966
		wool,	<2-4 "	"	"	
		cotton,	<1-2 "	78	"	
		wool	<2-4 "	"	"	
Polio Type 2	Tissue culture	cotton,	1-4 weeks	35	25	Dixon et al. 1966
		wool,	20 "	"	"	
		cotton,	2-10 "	78	"	
		wool	10-12"	"	"	
Vaccinia	Tissue culture	glass, vinyl, asbestos, ceramic tile, stainless steel	<5 weeks	3-96	25-37	Mahl & Sadler 1975
Polio Type 2	Tissue culture	"	1 day - 8 weeks	"	"	"
Coxsackie B3	"	"	2 weeks	"	"	"
Adenovirus 2	"	"	3-8 weeks	"	"	"
Herpes simplex 1	"	"	8 weeks	3-7	"	"
			4-24 hours	55-96	"	"
Coxsackie B3	Tissue culture	plastic, glass & paper	24 hours	ambient	6-37	McGeady et al. 1979

Table 1. (continued)

VIRUS	SOURCE OR SUSPENDING MEDIUM	SURFACE	SURVIVAL DURATION	ENVIRONMENT RH (%)	ENVIRONMENT TEMP (°C)	REFERENCES
Respiratory Syncytial Virus	Tissue culture & nasal secretions	formica, rubber gloves, cloth, paper tissue, skin (hands)	30 minutes to 8 hours	35-50	22-25	Hall et al. 1980
Rhino Type 2	Nasal washings & tissue culture	spoons, stainless steel, enamel, ball point pens, plastic, skin	7 days 24 hours " " "	ambient	23	Reed 1975
Rhino Type 2 & 14	Tris (10^{-3}) buffer, or buffer + bovine serum albumin, or saline	polyethylene vials (wet & dry)	24 hours	ambient	6, 23 & 37	Reagan et al. 1981
Influenza A (PR8 strain)	Suspension of infected mouse lung	glass cotton dust	6 weeks 2 weeks 2 weeks	ambient (in dark)	ambient	Edward 1941
Influenza A & B	Tissue culture	stainless steel, plastic cotton, paper, (transfer to hands)	24-48 hours (non-porous) <8-12 hours (porous) up to 24 hours from non-porous surfaces	35-56	26-29	Bean et al. 1982
Para-influenza 1, 2, & 3	Tissue culture in 0.5% gelatine	plastic petri dish	1-12 days 1-17 "	inside outside	21 -22-+33	Parkinson et al. 1983
Hepatitis B	Dried blood	stainless steel, cotton	14 days	42	25	Favero et al. 1974

Table 1. (continued)

VIRUS	SOURCE OR SUSPENDING MEDIUM	SURFACE	SURVIVAL DURATION	ENVIRONMENT RH (%)	ENVIRONMENT TEMP (°C)	REFERENCES
Hepatitis B	Plasma positive for HBV Markers	silanysed tubes & stainless steel	>1 week	42 (dark)	25	Bond et al. 1981
Hepatitis A	Faeces	glass vials	30 days	42	25	McCaustland et al. 1982
Herpes simplex	Saliva	skin, plastic, cloth	2.5-hours 4.0 hrs 3.0 hrs	ambient	ambient	Turner et al. 1982
Herpes Type 2 (MS strain)	Tissue culture	polystyrene petri dish (not dried)	4.5 hrs	Humid	37-40	Nerurkar et al. 1983
Herpex Simplex Type 1 & 2	Genital Secretions	gauze, gloves, speculum, plastic. contact transfer	72 hours 1-24 hours " " "	ambient	ambient	Larson & Bryson 1985
Rhino Type 39	Tissue culture & nasal secretions	formica, stainless steel, wood, 8 different fabrics, paper tissue, plastic petri dish	3 hours " " 1 hour 72 hours	ambient	23	Hendley et al. 1973
Human rota	Faeces	glass slides & cotton wool	12->35 days 12-45 days	13-92 92-94	20 4-37	Moe & Shirley 1982
Simian rota (SA11)	Faeces (rota -ve)	countertop	60 minutes	ambient	ambient	Keswick et al. 1983a

environmental surfaces and fomites as vehicles in the spread of viral infections. The summary of available information presented in Table 1 clearly shows that several types of viruses can survive well on human skin as well as a variety of fomites and environmental surfaces. However, the precise mechanisms by which transfer of viruses is brought about by these vehicles are yet to be established. Moreover, in only a very few instances has the actual recovery of the viral agent from the incriminated environmental surfaces or fomites been attempted. To a large degree, this is due to the general lack of availability of effective methods for the virological examination of such samples. Consequently, only a limited amount of work has been carried out to elucidate the true role and relative importance of these vehicles in the transmission of viral infections. Figure 1 shows the relative position of environmental surfaces and fomites in the cycle of spread of virus diseases.

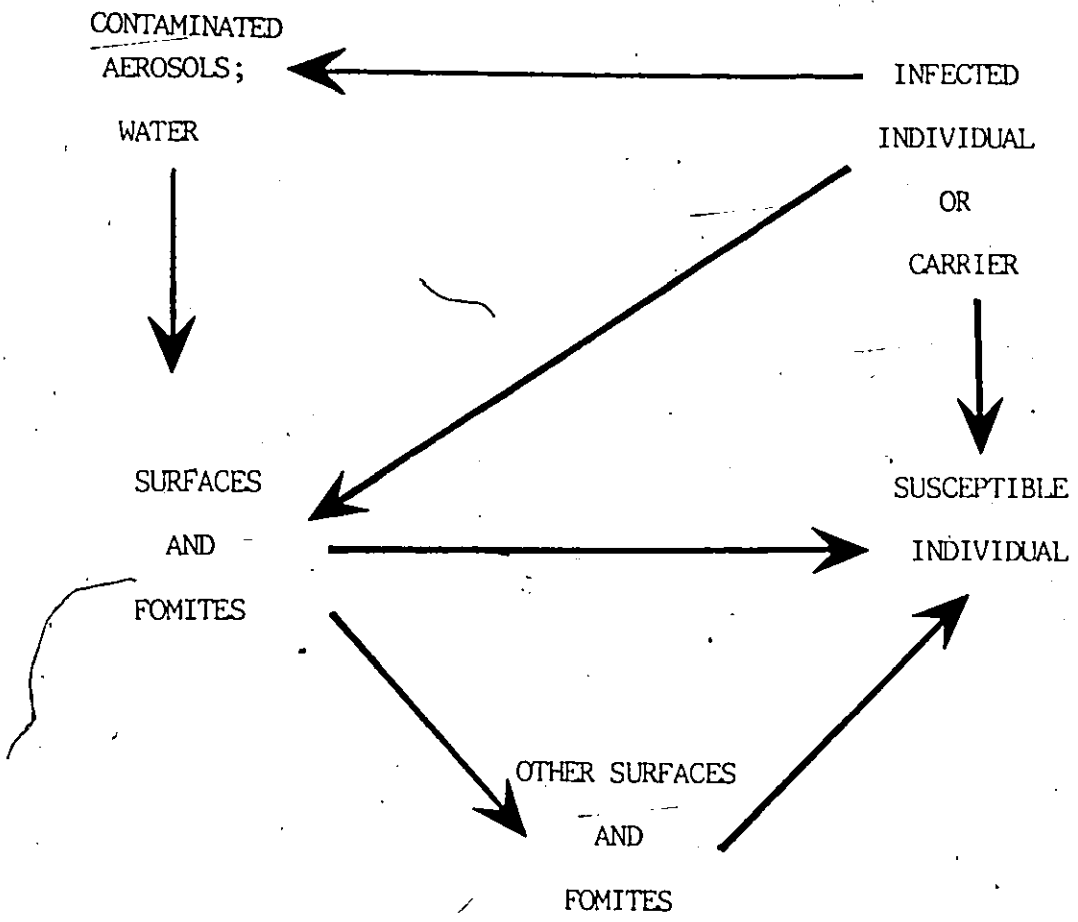
Around the turn of this century, investigations into the epidemics of smallpox in England showed that clothing and bed linen from infected persons were among the vehicles for the spread of this disease (MacCullum et al., 1957; Downie et al., 1965; Fenner & White, 1970; England, 1982).

Although the spread of hepatitis B virus (HBV) occurs principally through transcutaneous introduction of virus-contaminated blood or blood-based products (Bond, 1977; West, 1984), there is now evidence to suggest that fomites and environmental surfaces may also play a role in the spread of this infection. The fact that a wide variety of items or environmental surfaces may be involved in this context, is illustrated by the following two reports:

Investigations of an outbreak of HBV in a clinical laboratory (Pattison et al., 1974), incriminated the handling of computer cards, used

Figure 1

POSSIBLE ROUTES OF SPREAD OF VIRAL DISEASES



to request laboratory analysis, as the vehicle of transfer of the virus in the outbreak. Statistical analysis of risk factors showed a significant correlation between infection and a history of sustaining cuts while handling such laboratory requisitions. The implementation of preventative measures led to the termination of the outbreak.

Ringertz and Zetterberg (1967), reported an annual outbreak of HBV infection among trackfinders in Sweden. They described how the bare-legged runners sustained cuts and scratches from brushes as they ran, followed by communual use of washing facilities and towels at the end of the race. Mandatory use of protective leg clothing by all participants stopped further outbreaks.

In certain developing areas of the world exudates from ulcers have been found to have high levels of HBV (Foster et al., 1984). Contamination of the environment with such material is believed to be a likely source of the virus for susceptible persons in the surroundings.

It is generally believed that intimate person-to-person contact is required for transmission of herpesviruses since these agents are thought to lose their infectivity rapidly on drying. Therefore, it is assumed that fomites and surfaces are not potential vehicles of herpesvirus spread. However, now there is some evidence to suggest that these viruses could retain their infectivity for several hours on certain types of inanimate surfaces (Nerurkar et al., 1984; Larson & Bryson, 1985). This may explain the reports of nosocomial spread of herpesvirus infections (Linnemann et al., 1978).

Many viruses causing respiratory infections in humans are traditionally thought to spread only by the aerial route. However, studies carried out in the past 12 years show that environmental surfaces, fomites

and hands of infected individuals also play an important role in this regard.

Studies with rhinoviruses have shown that naturally-occurring and laboratory-adapted strains of these agents can be recovered from animate and inanimate surfaces several hours after the initial contamination had occurred (Gwaltney, Moskalski & Hendley 1978; D'Allessio et al. 1976). Experiments with human volunteers have also demonstrated that self-inoculation with virus particles from such surfaces could lead to the spread rhinovirus colds (Hendley, Wenzel & Gwaltney, 1973).

The data generated by Hall et al. (1975) suggests that nosocomial spread of respiratory syncytial virus (RSV) occurs via fomites and environmental surfaces and showed that under ambient conditions this virus could survive on such materials for at least a few hours (Hall, Douglas and Geiman, 1980).

D'Angelo et al. (1981) and Keenlyside et al. (1983) have described outbreaks of adenovirus keratoconjunctivitis associated with ophthalmology clinics. In both cases, the incriminated virus or its antigens were detected in samples collected from the environment inside the clinics.

In an outbreak of conjunctivitis among Vietnamese refugees in Guam (Arnow et al., 1977), enterovirus 70 (EV70) and adenovirus type 11 (AV 11) were both implicated. Infectious particles of EV70 and AV 11 were isolated from the towel and fingertips of patients, respectively.

The information summarized here shows that virus-contaminated fomites and environmental surfaces could act as vehicles for the transmission of a variety of viral infections. It also points to the importance of proper disinfection and antisepsis in the prevention and control of virus disease spread via such vehicles.

ROTAVIRUSES AND ACUTE GASTROENTERITIS IN HUMANS

The genus Rotavirus is a part of the family Reoviridae and consists of several antigenically related and morphologically identical viruses. These viruses are now known to be among the major causes of acute gastroenteritis in humans as well as a variety of animals (Cukor & Blacklow, 1984; Estes, Palmer & Obijeski, 1982).

Description

Rotaviruses are icosahedral in shape, with a particle diameter of 65-75 nm. The double-capsid structure gives the virus particles the distinctive wheel-like appearance (Flewett 1974). The core contains 11 segments of double-stranded RNA. In cesium chloride (CsCl), double-shelled particles of rotaviruses have a density of $1.36 g/cm^3$. The outer capsid is believed to be essential for virus infectivity (Bridger & Woode 1976; Elias 1977), because its removal by calcium chelation renders the virus particles non-infectious (Cohen et al., 1979).

Rotaviruses and acute gastroenteritis

Human rotaviruses (HRV) were first described as aetiological agents of gastroenteritis by Bishop et al. (1973). Although the disease occurs in other age groups, children 6 to 24 months of age are most frequently affected. The incubation period for rotaviral gastroenterits is usually 1 to 3 days. The disease is characterised by severe diarrhoea, vomiting and fever (usually low grade). Symptoms generally last for 5 to 8 days. Severe dehydration can occur in up to 83% of cases and be life-threatening or the the cause of death in malnourished children (Carlson et al., 1978; Black et al., 1981; Mata et al. 1983; Rowland et al. 1984). Fatal dehydration has also been reported in geriatric patients (Marrie et al. 1982). The stools are usually watery, sometimes with mucus and fatty material, but without

blood. Very large numbers of rotavirus particles (>10^{10}/gram) are often found in the faeces of infected individuals (Flewett, 1983; Davidson et al., 1975). In neonates rotavirus shedding may occur as early as the first day of birth, but such infections are frequently asymptomatic (Murphy et al., 1977; Perez-Schael et al., 1984).

As was stated earlier, rotavirus gastroenteritis is by no means restricted to children. There are several reports of the disease in the adult populations (Elias, 1977), particularly in the more developed parts of the world. Health care attendants (Hoh, Presser & Wigand, 1983), institutionalised elderly persons (Cubitt & Holzel, 1980; Halvorsrud & Orstavik, 1980), staff in day-care centres (Lemp et al., 1984) and adults whose children have rotavirus infection (Wenman et al., 1979; Grimwood et al., 1983) appear to be at a higher risk.

Apart from overt cases of diarrhoeal disease, rotaviruses cause a large number of cases of asymptomatic infection (Murphy, Albrey & Crewe, 1977; Wenman et al., 1979; Rossi et al., 1982; Holdaway et al., 1982a; 1982b; Champsaur et al., 1984a, 1984b). Such individuals pose a significant hazard to others in their immediate surroundings. This is particularly important in institutional settings where relatively crowded conditions and use of common facilities may promote virus transmission. Keswick et al. (1983b) have suggested that the prevalence of asymptomatic rotavirus shedding in day-care centres make them a reservoir of infection for previously uninfected children and their family contacts.

Although gastroenteritis remains the only clinical condition definitely associated with rotaviruses, these agents or their antigens have been detected in cases of intussusception (Konno et al., 1978), central nervous system involvement (Salmi, Arstila & Koivikko, 1978), sudden infant death syndrome (Yolken & Murphy, 1982), otitis media (Klein et al., 1982)

and pneumonia (Santosham et al., 1983).

Epidemiology of human rotavirus gastroenteritis

Rotaviruses have been implicated in outbreaks of acute diarrhoea in the general community (Freij et al., 1978; Lycke et al., 1978; Morens et al., 1979; Echeverria et al., 1983), but these viruses are particularly important for their capacity to cause frequent outbreaks of acute gastroenteritis in institutions such as hospitals (Ryder et al., 1977; Murphy, Albrey & Crewe, 1977; Middleton, Szymanski & Petric, 1977; Holzel et al., 1980; Hoh, Presser & Wigand, 1983), nursing homes (Halvorsrud & Orstavik, 1980; Marrie et al., 1982), day-care centres (Haug, Orstavik & Kveldstad, 1978; Pickering et al., 1981) and schools (Hara et al., 1976; Sutmoller et al., 1982).

A striking feature of outbreaks of rotavirus gastroenteritis is their seasonality. In regions with a temperate climate, outbreaks of rotavirus diarrhoea generally occur in winter (Bryden et al., 1974; Davidson et al., 1975; Middleton, Szymanski & Petric, 1977; Brandt et al., 1982; Konno et al., 1983). The data available from certain tropical areas show an increase in the number of cases of rotaviral gastroenteritis in the cool and dry months of the year (Maiya et al. 1977; Black et al. 1981; Mata et al. 1983; Goh Rowland et al., 1985).

Transmission of rotaviral gastroentritis

The spread of rotaviral infections in nature is believed to occur mainly through the faecal-oral route. Kapikian et al. (1983), using human volunteers, have shown that ingestion of viable rotavirus particles results in infection. However, the true role and the relative importance of various types of vehicles in the transmission of rotaviral infections has not yet been determined.

Rotaviruses of human origin have been shown to survive for prolonged periods in water (Raphael, Sattar & Springthorpe, 1985), air (Ijaz et al., 1985b) and on glass slides and cotton swabs (Moe & Shirley, 1982). Consumption of sewage-contaminated drinking water is also believed to have resulted in outbreaks of rotaviral diarrhoea (Hung et al., 1984). Although air is also suspected as a vehicle in rotavirus spread (Foster et al. 1980; Brandt et al. 1982), documentary proof to substantiate this view is still lacking. However, there is some evidence to suggest that faecally-contaminated hands and environmental surfaces may act as vehicles in the spread of rotaviral infections (Halvorsrud & Orstavik, 1980; Noone & Banatvala 1983). This view is further substantiated by: a) the ability of these viruses to survive for several days outside the human host (Moe & Shirley, 1982), b) the recovery of infectious rotavirus particles from articles of common use in a day-care centre (Keswick et al., 1983b), c) the detection of rotavirus antigens in handwashings of attendants of patients with rotavirus diarrhoea (Samadi et al., 1983) and d) the rapid spread of the disease by clustering, close contact or interaction with infected persons (Holzel et al., 1980; Steinhoff & Gerber, 1976).

CHEMICAL DISINFECTION OF SURFACES

An important weapon in the fight against communicable viral diseases is chemical disinfection. The primary function of products recommended for surface disinfection is to render the viral pathogen irreversibly non-infectious on the contaminated surface and thereby interrupt the chain of transmission.

The two basic systems used for the determination of the virucidal capacity of chemical disinfectants and antiseptics are the "suspension" test and the "carrier test". The former involves the mixing of the test

virus with appropriate dilutions of the product under evaluation. This is followed by immediate dilution of the mixture at the end of the required contact time, to a level sufficiently high to terminate the action of the product. In the "carrier" test, experimentally-contaminated surfaces or objects are exposed to the product being assessed; at the end of the contact time, the reaction is terminated as above, the virus is eluted from the treated surface and assayed for infectivity. The carrier test is thus more closely related to the in-use practices of general surface disinfection. A product is regarded as an ideal virucide only if it is able to inactivate a virus both in suspension and on contaminated surfaces.

The suspension test has limited applicability because in general, it can only be considered relevant to the disinfection of drinking and waste waters and the decontamination of virus suspensions in laboratory settings. Nevertheless, the vast majority of reported tests for virucidal disinfectants have been done in suspension because this type of test is relatively easy to conduct compared to the carrier test. In the latter, it is important to determine the quantity of contaminating virus which is removed by the elution procedure before an accurate measure of virus inactivation can be made. The suspension test is, therefore, extremely useful as a prescreening tool to determine which disinfectants may also be effective on contaminated surfaces. However, as summarised in Table 2, relatively few studies have been done on the chemical disinfection of virus-contaminated surfaces and the wide variations in test protocols used by the different investigators make it difficult to draw any general conclusions. Moreover, many of the chemical disinfectants in common use have not been properly tested for their virucidal activity.

The efficacy of the virucidal process is strongly influenced by seve-

Table 2

DISINFECTION OF VIRUS-CONTAMINATED SURFACES : EXPERIMENTAL STUDIES

DISINFECTANT COMPOSITION	SURFACE TESTED	VIRUSES STUDIED	SUSPENDING MEDIUM	CONTACT TIME	METHOD OF ELUTION	COMMENTS	REFERENCES
Alkalinized glutaraldehyde (2%)	stainless steel cylinders	polio 1 & 2, coxsackie B1, ECHO 6, HSV, vaccinia, adenovirus 2, mouse hepatitis.	tissue culture fluid (TCF)	10 mins	Immersion	Prevented the multiplication of all viruses	Borick et al. (1964)
Quaternary ammonium (5), Bromosalicil-anilides (2)	wool gabardine, wool blanket, cotton	vaccinia, polio	TCF	16 hours	maceration of fabrics in TCF	Marked decrease in vaccinia virus persistence on fabrics impregnated with 3 quats. All ineffective against polio. Vaccinia persisted <1 days & polio <5 days on triazone finished fabrics.	Sidwell et al. (1967)
A quaternary ammonium (quat.) A phenolic, & Alkalinized glutaraldehyde, Anionic & a Cationic detergent	woolskin pads, leather pads	vaccinia polio	TCF	10 mins wash & 3 mins rinse	homo-genised	Vaccinia titres reduced in all experiments, & undetectable in glutaraldehyde/ detergent combinations. Polio not inactivated, present in high titres in rinse water.	Sidwell et al. (1970)
H_2O_2, chloramine, hexylresorcinol	surfaces & air	adeno 3, polio 3, coxsackie 1	?	30 mins	?	Different concentrations were needed to inactivate each virus. Higher for surfaces than air.	Slobodenyuk et al. (1970)
Phenols, Formic acid, Formaldehyde Peracetic acid, and Chlorinated lime.	surface & air	Newcastle disease virus	allantoic fluid/ serum	30 mins 24 hours		Peracetic acid & phenolics were active at all temps. Formic acid and chlorines slightly at low. Formaldehyde also slow at 0°C & ineffective below -10°C.	Stellmacher et al. (1973)

Table 2 (continued)

DISINFECTANT COMPOSITION	CARRIER TESTED	VIRUS STUDIED	SUSPENDING MEDIUM	CONTACT TIME	METHOD OF ELUTION	COMMENTS	REFERENCES
A quat. ammonium, A phenolic, An iodophor	stainless steel cylinder	HSV, polio 1, vaccinia	TCF	10 mins	rinse	The iodophor was most effective. Quat & phenolic comparable in effect but could not cause a 3log$_{10}$ drop in titre of of polio.	Gaustad et al. (1974)
Formaldehyde vapour	petri dish	avian adenovirus (TR-59)	TCF	24 hours (15-25°C 100% RH)	rinse	virus was completely inactivated	Furuta et al. (1976)
Glutaraldehyde 2% Formaldehyde 2% Phenol 5%, Chloramine T 2%, Alcohols 60-80%	scabs	vaccinia	scabs	1.5 - 3.0 hours	?	Virus inactivated by most disinfectants when scab immersed.	Schumann et al. (1977)
Chloramine T 1.5%, Isopropanol 70%	hand	vaccinia	?	2-5mins	?	Chloramine T and isopropanol caused complete inactivation when tested on hands.	
"Gigasept" 5% (succindi-aldehyde & formaldehyde)	plastics, rubber aluminium	coxsackie type B3	TCF " gelatin	30-240 mins	rinse	Optimum virus disinfection after 4 hours.	Thraenhart & Kuwert (1977)
Alcohols >40%, Iodophor (iodine 1%) Hexachlorophene plus alcohol, Quat. ammonium	hand & finger -tips	rhino 29 & 39	TCF	Few seconds, with 1 hour residual effect of iodine	rinse	1% iodine most effective followed by a combination of the other products when suspended in foam.	Hendley et al. (1978)
Ethanol, Iodines (2), Phenolics (3), Quat. ammonium compounds (2), 2% glutaraldehyde, chlorohexidine, sodium hypochlorite, sodium hydroxide	petri dish & wet wool	porcine parvo-virus (PPV), pseudorabies virus (PRV), transmissible gastroenteritis virus (TGV)	TCF	5-20 mins	rinse	PRV & TRV were inactivated by all disinfectants tested, but only sodium hydroxide & sodium hypochlorite inactivated PPV in the 5 min., and 2% glutaraldehyde & double-strength formaldhyde in 20 minutes.	Brown (1981)

Table 2 (continued)

DISINFECTANT COMPOSITION	CARRIER TESTED	VIRUS STUDIED	SUSPENDING MEDIUM	CONTACT TIME	METHOD OF ELUTION	COMMENTS	REFERENCES
Formaldehyde	filter paper, fibre glass, aluminium foil	polio 3, vaccinia & ECHO 14	TCF & serum	overnight	rinse	Polio highly resistant. Vaccinia & ECHO inactivated. Filter paper unsuitable carrier. Fumigation ineffective. Organic material protects virus.	Everall et al. (1982)
Ethanol 78.2% Isopropanol 1% Bromomethyl phenol).1%	hand & fingertips	fowl plague, influenza A, vaccinia, adenovirus 5, polio 1 & 2, coxasackie B3 & B4, ECHO	serum	10 mins	rinse	enveloped viruses more susceptible to alcoholic agents than naked ones. Suspension test predicted a greater effectiveness than observed in vivo.	Schurmann et al. (1983)
Sodium hypochlorite, 2% aqueous glutaraldehyde, 2% glutaraldehyde plus 7% phenol, 70% isopropanol, iodophore detergent (80ppm I_2)	silicon-ised tubes	hepatitis B virus	dried plasma	10 mins at 20°C	swab-rinse	Virus infectivity destroyed by all. HBV not as resistant to disinfectants of intermediate to high activity.	Bond et al. (1983)
methylated spirits, iodine/iodate, hypochlorites	swine skin hands	porcine enterovirus poliovirus types 1,2,3	faeces faeces	10 mins	rinse	Methylated spirits ineffective, iodine had marked activity but some infectious virus remained, 20% bleach was effective but caused skin irritation, lower concentrations ineffective	Cliver & Kostenbader (1984)
2% glutaric acid	hands	rhinovirus serotype 2	TCF	10 mins to 6 hours	rinse	Lotion had long-lasting effect but reduced with physical activity.	Hayden et al. (1984)
Combination of citric acid, malic acid and sodium lauryl sulphate.	paper tissue	rhinovirus (8 strains of different serotypes)	TCF & nasal mucus	3 to 5 seconds	none	Pronounced reduction in virus infectivity but not against all serotypes. Virucidal effect not residual and overcome by large inoculum.	Hayden et al. (1985)

ral factors including the nature of the virus, the surface to be disinfected, the type of disinfectant and the environmental conditions under which the procedure is taking place. Hence, in addition to the virus involved, any appropriate testing protocol should take the following factors into consideration (Chen & Koski, 1983; Grossgebauer, 1970):

(1) the nature of the medium in which the test virus is suspended
(2) the nature of the diluent used to bring the disinfectant to its recommended working strength
(3) the choice and experimental contamination of the surface to be employed as the virus carrier
(4) the length of time the virus is exposed to the disinfectant
(5) the procedure for stopping the disinfectant action immediately after the contact time is over
(6) the procedure for making the disinfectant non-toxic for cell cultures

The nature of the virus suspending medium

Apart from physical protection of the virus, an organic or inorganic load may specifically inactivate an applied disinfectant. Such a phenomenon is well documented in the case of nitrogenous substances such as proteins and a variety of disinfectant types (Chen & Phebus, 1978). The ideal organic load for testing virucidal efficacy of disinfectants is the body secretion or excretion in which the virus might be expected to occur in nature. Therefore, use of purified virus in testing the virucidal efficacy of such products may give misleading results. However, frequently, for practical reasons, it is necessary to simulate an organic and inorganic load when using laboratory grown virus stocks. Substances such as foetal calf serum, faecal extracts and yeast cells (Evans et al., 1977) have been

used for this purpose.

The nature of the material used for disinfectant dilution

The nature of the diluent can markedly affect the virucidal activity of a disinfectant. In most cases disinfectants are diluted with tap water, especially in the absence of any specific instructions to the contrary. There are, however, instances where tap water is recommended by the manufacturer, but the actual testing of such products was carried out using distilled water as diluent (Drulak et al., 1978a). The use of distilled water as a diluent has in fact been shown to give results which overrate the virucidal potential of the products under test (Wallis et al., 1963; Sattar et al., 1983).

Contact time between virus and disinfectant

Depending on the application (for hard surface, instrument soaks, laundering or topical use), the reaction time between virus and disinfectant can vary from a few seconds to several hours. However, it is generally agreed that in order to declare a disinfectant as effective, it should be able to produce a pronounced virucidal effect after a minimal contact time. The summary (Table 2) of the available data on studies of the disinfection of virus-contaminated surfaces, clearly demonstrates the wide variability in virus/disinfectant contact time used by various investigators.

Procedure for terminating disinfectant action and for making the reaction mixture non-toxic for the cell culture assay system

Addition of substances such as skim milk (Drulak et al., 1978a; 1978b; Wallbank et al., 1978) or letheen broth (Russell et al., 1979) is generally used not only to stop the disinfectant action, but also to render the reaction mixture non-toxic for cell cultures. However, not all disinfec-

tants are inhibited by these substances. Dilution of the reaction mixture immediately at the end of the contact time is an alternative method of achieving the same aims. This is the method most commonly used for surface disinfection testing. It should be noted here that this dilution method necessitates the use of high titres of virus in order to be able to demonstrate the generally accepted 3 $\log_{10}$ reduction in virus infectivity titre.

Chemical disinfection of rotaviruses.

There are as yet no published reports on the chemical disinfection of rotavirus-contaminated fomites and environmental surfaces. All previous reports on rotavirus disinfection are based solely on suspension tests using mainly animal rotaviruses. A summary of the available information from these studies is given below.

In a preliminary study, Snodgrass and Herring (1977) tested the action of four disinfectants on lamb rotavirus. When faecal suspensions of the virus were used, only 10% formol-saline and 5% Lysol were able to inactivate most of the virus after a 2-hour contact time. 4% FAM (an iodophore), and 3% Chloros (a sodium hypochlorite solution with 11% available chlorine) were unable to inactivate the virus even after 2 hours of contact. The amount of available chlorine in the hypochlorite solution tested here was many times that used in the routine disinfection of drinking water and sewage.

Kurtz, Lee & Parsons (1980) tested the efficacy of methanol, ethanol, propan-1-ol, propan-2-ol and butan-2-ol against calf rotavirus. All except methanol were able to bring about a 3-4 $\log_{10}$ reduction in virus titre when used as 30-40% solutions and with a contact time of only 1 minute. A 50% solution of methanol was required to produce the same effect. Presence of

faecal matter in the virus suspending medium appeared to have very little effect on the efficacy of these alcohols as disinfectants.

Tan and Schnagl (1981) tested the effect of a variety of disinfectants on simian rotavirus SA-11 in the presence or absence of faecal matter. All the antiseptics tested (Betadine, Hexol, Hibiclens and Hibitane) were found to be relatively ineffective against this virus. Hibiclens was not able to completely inactivate the clarified virus even after 2 hours of contact, whereas the other 3 (Hexol, Betadine and Hibitane) required up to 30 minutes to inactivate virus suspended in faeces. Ethanol (95% v/v), which can be used for skin disinfection, and 5% Biogram (5% v/v; chlorinated phenolic compounds) were able to inactivate the virus after a 1 minute contact period, even in the presence of faecal material. Formaldehyde, as 10% or 4% solutions in saline, required from 5 to 30 minutes of contact time to completely inactivate the virus.

Undiluted Milton's antibacterial solution (1% sodium hypochlorite with nearly 11,000 ppm chlorine) required 15 minutes to inactivate clarified simian rotavirus (SA-11) and showed variable reactivity against SA-11 suspended in faecal matter. This disinfectant is recommended for use on baby bottles and teats, and a subsequent communication from these same authors (Tan & Schnagel, 1983) has reported a reappraisal of the Milton Antibacterial Solution using a "partially purified", laboratory-adapted strain of human rotavirus (Wa) suspended in phosphate buffered saline and an organic load of infant formula. Under these experimental conditions, both undiluted and 1/80 dilution of Milton Antibacterial solution was able to completely inactivate the rotavirus tested.

Brade, Schmidt & Gattert (1981) tested eight disinfectants against

simian rotavirus SA-11 and found that only Desderman (an alcohol based product) and Orbiphen (a phenolic compound) could produce a >3log$_{10}$ reduction in the virus titre with a minimum contact time of 1 minute. Both these disinfectants were effective even in the presence of an organic load of 20% foetal calf serum or a 0.1% soap solution.

Studies on the inactivation of SA-11 by chemical disinfectants used in hospitals and microbiology laboratories (Sattar et al., 1983; Raphael, Sattar & Springthorpe, 1983) have shown that only 7 of the 15 products tested in suspension could reduce the virus titre by more than 99.9%. Of those disinfectants shown to be effective, 2 (Sanarinse & Septisol), were hexachlorophene-based products (which are no longer widely available because of their toxicity to humans); Cidex (2% acid glutaraldehyde) is known to cause contact dermatitis and formalin is a potential carcinogen. Chlorine is probably the most widely used disinfectant, and a hypochlorite solution at 5,000 ppm free chlorine is also recommended as a general purpose virucide by the Medical Research Council of Canada (1980). Here it should be noted that one of the products found to be ineffective was Javex used at a concentration of 5,000 ppm free chlorine.

There has been some recent interest in the use of peracetic acid as a disinfectant which does not produce toxic by-products. However, the concentrations necessary for the inactivation of SA-11 and a human rotavirus, as well as enteroviruses, preclude its use as a general purpose virucide (Harakeh, 1984). Apart from the above paper, the only other previously published work on disinfection of human rotaviruses (Narang & Codd, 1983) was based solely on morphological disruption of the virus particles as viewed by electron microscopic examination of virus-disinfectant mixtures. This cannot reliably distinguish between infectious

and non-infectious virus particles.

In studies recently completed in this laboratory, Springthorpe et al. (1986), using the suspension test, have examined over 70 disinfectant products, for their capacity to inactivate HRV in the presence of varying levels of organic load. The results obtained, showed that most of these chemical disinfectants, which are in common use in the institutional, domestic and work environment are ineffective in the inactivation of HRV particularly in the presence of organic matter.

MAIN OBJECTIVES OF THE STUDY

The main objectives of this study were as follows:

1) to develop a simple and effective modification of the "rinse" method for recovery of virus from contaminated surfaces.

2) to study the survival of human rotavirus on a variety of inanimate surfaces in order to determine their potential to act as vehicles in the transmission of rotaviral infections.

3) to ascertain the influence of different types of surfaces on survival of human rotavirus.

4) to determine whether environmental factors such as humidity and temperature could promote or retard such virus survival.

5) to develop a simple and efficient protocol for the assessment of chemical disinfection of non-porous inanimate surfaces.

6) to evaluate the suspension test for virus disinfection as a reliable pre-screening tool for the chemical disinfection of virus-contaminated surfaces.

7) to identify which classes of chemical disinfectants are capable of efficient disinfection of rotavirus-contaminated non-porous surfaces.

MATERIALS AND METHODS

Cells

The MA-104 line of embryonic rhesus monkey (Macaca mulatta) kidney cells (Microbiological Associates Inc. Bethesda, Md., U.S.A.) was used throughout this study. Our original seed culture of this cell line was supplied to us by courtesy of Dr. H. Malherbe (Gull Laboratories, Salt Lake City, UT, U.S.A.). The cells were grown at 37°C in 75 cm^2 culture flasks (Costar, Cambridge, MA, U.S.A.) in minimal essential medium (MEM; AutoPow, Flow Laboratories, Rockville, MD, U.S.A.). Every 450 mL of MEM was buffered with 10.8 mL of a 5.6% solution of sodium bicarbonate and supplemented with 200 mM glutamine and 5% (v/v) foetal calf serum (FCS; Bockneck, Mississauga, Ont.) The medium for maintaining already formed cell monolayers contained only 2% FCS, but was otherwise identical to the growth medium. This cell line was passaged twice a week at a split ratio of 1:3 using 0.005% (w/v) trypsin (VMF trypsin; Millipore Corp. Bedford, MA, U.S.A.) in a 0.02% (w/v) versene solution. The passage history of the cell line before its arrival in our laboratory was not available. However, during this study the cells were subjected to 120 passages.

Cell monolayers for the determination of the virus plaque forming units (PFU) were prepared in 12-well plastic cell culture plates (Costar, Cambridge, MA, U.S.A.). Approximately 5×10^4 viable cells suspended in 2 mL of growth medium were added to each well and the plates incubated at 37°C in a 5 % CO_2 atmosphere. Within 48 h, the cell monolayers were ready to be used for plaque assay.

Unlabelled virus

A cell culture-adapted strain Wa (Wyatt et al. 1980) of human rotavirus (HRV), kindly supplied to us by Dr. R.G. Wyatt (U.S. National Insti-

tutes of Health, Bethesda, MD, U.S.A.), was used in this study. The virus was plaque purified once in our laboratory and, for the preparation of the virus pools to be used here, each monolayer in a 75 cm^2 culture flask was inoculated with 0.5 mL of the virus to give a multiplicity of infection of 1 : 100. The inoculated monolayers were then maintained in MEM (without serum) supplemented with 5.0 ug/mL trypsin (NBC). Complete cytopathic degeneration of the monolayers generally occurred within 72 h of incubation at 37°C. The infected cultures were frozen (-70°C) and thawed 3 times to aid virus release. The virus suspension was clarified of cellular debris by centrifugation at 1,000 x g at 4°C, for 15 min. The virus in the supernatant was subsequently concentrated 10X by ultracentrifugation at 100,000 x g for 120 min. The pelletted virus was resuspended in tryptose phosphate broth (TPB; Difco, Detroit, MI, U.S.A.), prior to plaque assay and subsequent storage at -70°C.

Radio-labelled virus

^{14}C-labelled HRV was prepared as follows. Two-day old monolayers of MA-104 cells in 490 cm^2 plastic roller culture flasks (Corning Glass Works, Corning, NY, U.S.A.) were washed to remove all traces of FCS and were starved overnight in Earle's balanced salt solution (EBSS) containing 1/20th the concentration of amino acids and vitamins usually present in MEM. The cells were then infected and, after a virus adsorption period of 3 h, maintained in EBSS supplemented with 1/20th the normal MEM amino acid cand vitamin concentrations, 1.0 uCi/mL of L-[U-^{14}C] amino acids (Amersham, Oakville, Ont.), 0.1 uCi/mL D-[U-^{14}C] glucosamine (Amersham) and 5.0 ug/mL of trypsin (NBC). After 72 h incubation, when >90% of the cells had lysed, the labelled virus was harvested and concentrated as described above.

The resultant virus pellet was then resuspended in tryptose phosphate

broth (TPB) and purified on an isopycnic cesium chloride (CsCl) gradient. To achieve this, the virus concentrate was first suspended in a 40% (w/v) solution of cesium chloride, and subsequently layered on to a second solution of CsCl prepared at a higher concentration (55% w/v), and centrifuged at 100,000 x g for 20 h at 4°C. A series of fractions was then collected starting from the bottom of the tube, using a Varioperpex peristaltic pump (LKB-Produkter AB, Bromma 1, Sweden). The refractive index and radioactivity of each fraction was measured. Those fractions corresponding to the density of double-shelled rotavirus particles were pooled and dialysed to remove the CsCl and any remaining low molecular weight ^{14}C-labelled material. Plaque titres of these pools were equivalent to that of the unlabelled virus.

Virus quantitation by plaque assay

In the plaque assay for infectious virus (Ramia & Sattar, 1979), the cell monolayers were thoroughly washed with EBSS to remove all traces of FCS. A 0.1 mL volume of the appropriate dilution of the sample was inoculated into each of three wells and incubated at 37°C for 1 h to allow for virus adsorption to the cells. The monolayers were maintained under an overlay of MEM containing 0.6% agarose (Type II; Sigma Chemical Company, St. Louis, MO, U.S.A.) and 5.0 ug/mL trypsin (Nutritional Biochemical Corporation, Cleveland, OH, U.S.A.), the plates sealed into plastic bags (Philips Electronics Ltd., Scarborough, Ont.) and incubated at 37°C. Countable plaques were usually present after 72 h. The cell sheets were fixed in 10% formol saline for not less than 2 h and stained with a 0.1% aqueous solution (w/v) of crystal violet.

Faecal samples

In order to simulate as closely as possible the conditions generally encountered in nature, the virus to be used here was suspended in samples

of faeces obtained from laboratory-confirmed cases of rotaviral diarrhoea at the Children's Hospital of Eastern Ontario (CHEO), Ottawa, Ont. Four such samples were tested in this study and each one of these was first prepared as a 10% (w/v) suspension in normal saline, and centrifuged at 1,000 x g for 15 min to remove large particulate matter. The indigenous rotaviruses and other enteric viruses that may also be present in these stools could not produce countable plaques in our assay system. Therefore, they did not appear to interfere with the quantitation of the experimentally-added rotavirus.

Surfaces tested as carriers

The following types of materials were selected as representative of porous and non-porous inanimate surfaces found in areas of domestic and institutional settings where HRV contamination is most likely to occur. In addition, for the evaluation of chemicals for surface disinfection a "carrier" test is generally required and used in this assessment (Klein & Deforest, 1983). In this test the product under evaluation is applied to an experimentally-contaminated virus carrier and, after the required contact time, the virus is eluted to measure the loss in its infectivity. The four types of non-porous inanimate carriers selected also represent surfaces commonly subject to chemical disinfection.

Non-porous materials: The non-porous inanimate carriers tested were, (a) glass, (b) stainless steel, (c) rough plastic (Milar) and (d) smooth plastic (vinyl).

Glass cover slips (Chance Propper Ltd., Warley, England) of 1 cm diameter were purchased from Johns Scientific (Mississauga, Ont.). Sheets of the other three types of materials were obtained from local retail outlets and disks of 1 cm diameter were cut from them. Prior to contamina-

tion with the virus suspension, the disks were cleaned by sonication for 10 min in a 2% 7X cleaning solution (Linbro, Flow Laboratories) followed by thorough rinsing in running deionised water. They were then soaked for 10 min in 95% ethanol and air dried before being placed individually in the wells of a 24-well plastic cell culture plate (Costar); cleaned disks were handled only with a vacuum pick up device or a pair of sterile forceps.

Porous materials: One cm diameter pieces of the following types of porous materials were tested in this study: (a) cloth (cotton-polyester) from a used and laundered isolation room gown obtained from the CHEO, (b) Canadian paper currency as a new one dollar bill, (c) white poster card purchased at a local retail outlet, (d) sheets of 100% cotton parchment and white bond paper available in the department and (e) paper from a bedside temperature recording chart and a virological test requisition form obtained from the CHEO.

Eluents tested for the recovery of virus from the surfaces

The following were evaluated as possible eluents for the recovery of HRV from our carriers:

Deionised distilled water (DDW)

Phosphate buffered saline (PBS)

Earle's balanced salt solution (EBSS)

Tryptose phosphate broth (TPB)

1% bovine serum albumin (BSA)

3% arginine / 3%glycine (ARG/GLY)

Further details on these eluents are summarized in Table 3.

Procedure for elution of virus from the disks

Using a Gilson (Mandel Scientific Co. Ltd., Rockwood, Ont.) variable volume pipettor and disposable plastic tips, each disk was contaminated by placing on its surface 20 uL of the labelled virus suspended in faeces.

Table 3

RELEVANT INFORMATION ON VIRUS-ELUTING AGENTS USED IN THIS STUDY

Item	Manufacturer	Concentration used	pH
Tryptose phosphate broth (TPB)	Difco	1X in deionised water	9.0
Bovine serum albumin (BSA)	Sigma Chemical Company	1% in deionised water	9.0
Arginine (ARG)	Sigma Chemical Company	3% in deionised water	9.0
Glycine (GLY)	Sigma Chemical Company	3% in deionised water	9.0
Earle's balanced salts solution (EBSS)	Gibco	1X in deionised water	8.0
Phosphate buffered saline (PBS)	Laboratory prepared	in deionised water	7.4
Deionised distilled water (DDW)	Laboratory prepared		6.5

* pH adjustments where needed, were made with 1N NaOH.

All agents, except BSA & ARG, were sterilised by autoclaving.

BSA & ARG were sterilised by filtration and used as a mixture.

The inoculum was then allowed to air dry by keeping the disks for 2 h in a hood with a vertical laminar flow of HEPA-filtered air. A separate set of contaminated disks were similarly dried overnight in order to determine the effect of prolonged drying on virus recovery from the surface. A 1.0 mL volume of the eluent under test was then added to a set of 4 wells containing one each of the four carriers. Elution of the virus from the surface was achieved by sonication of the carriers for 10 min in the 24 well plate, placed in a sonic bath (Bransonic 52; O.H. Johns Scientific, Toronto, Ont.). The eluate in each well was then agitated by means of a P1000 Gilson Pipetman. The radioactivity associated with the eluted material was then measured in order to determine the efficiency of virus elution. All tests were conducted in triplicate.

Control of relative humidity and temperature

All HRV survival experiments, except where indicated, were carried out in a transparent glass chamber (21 x 36 x 55 cm^2). Virus survival was tested at low (25±5%), medium (50±5%) and high (85±5%) relative humidity (RH) levels. Air entering the chamber was passed through a tube containing a dessicant (Drierite; Hammond, Xenia, OH, U.S.A.) to obtain the low RH and it was humidified by bubbling it through deionised water contained in a gas-washing bottle to achieve the high RH. During the period of these experiments, the ambient RH always stayed within the mid-range (45-55%), and, as a result, did not require any further adjustments. However, in one experiment the RH was kept at the medium level in the chamber to confirm the results obtained under ambient conditions of RH.

To test the effect of temperature on virus survival, the chamber was held in a climate controlled room for mid-range temperature (22±2°C), in a cold room for low temperature (4°C). At the high temperature, experiments

were conducted in a walk-in incubator in which the air temperature was kept at 37°C and the ambient relative humidity remained stable at 36±1% throughout the experiments.

Test procedure for survival experiments

Each disk was contaminated with 20 uL of the faecal suspension containing approximately 10^7 PFU of the virus/mL. The inoculum was then allowed to air dry by keeping the plates with the disks for 2 hours in a vertical laminar flow hood. The amount of infectious virus remaining after this initial drying period was considered as the input virus level. The plates were then placed in the glass chamber held at the desired temperature. For the virus survival experiments at room temperature the chamber was placed in a room subject to diurnal cycles of fluorescent light. Air at the desired level of relative humidity (RH) was circulated through the chamber to give approximately six air changes per h. The air temperature and RH in the chamber were continuously monitored using a recording hygrothermograph (Cole-Parmer Instrument Co., Chicago. IL, U.S.A.) placed inside the glass chamber.

At the appropriate sampling times, disks of each type were transferred to separate glass vials, and 1.0 mL of TPB was added to each. To effect maximal virus recovery from the surfaces, these vials were then placed in a sonic bath (Bransonic; Johns Scientific, Toronto, Ont.) and sonicated for 10 min. The eluate was then further mixed using a micropipettor before plaque assay. For any given experiment on virus survival, samples were collected at least six different times during the observation period.

Not less than three replicate experiments were conducted at each of the three RH levels tested using the same faecal sample. In addition, experiments were performed with three other faecal samples to establish whether virus survival was independent of the faecal sample used for sus-

pending the virus.

To determine the effect of changes in RH on virus survival, a separate set of experiments was conducted. Following the above-mentioned protocol, disks were shifted between mid and high RH levels during the experiments.

Statistical analysis

Virus inactivation curves were plotted, and linear regression analysis (Colton, 1974), on the log scale, of these gave an inactivation coefficient (Ki) of $\log_{10}$ reduction in virus titre/day for each set of experimental conditions. The Student "t" test (two-tailed) was used to compare the effect of surface type on rotavirus survival. Subsequently, the effect of relative humidity and temperature was determined using the Ki values and compared using the "t" test (Appendix III).

Disinfectants

Twenty-seven different disinfectant products and formulations were tested in this study. The selection was based on their performance in the suspension test (Springthorpe et al., 1986), the manufacturer's recommended use for surface disinfection, and/or the nature of their active ingredient(s). Table 7 (page 50), shows the products tested in the form of a numbered code, and their listed ingredients by a three lettered code. The key of this code is given in Appendix 1.

The data generated from these disinfectant experiments (Table 7), are presented utilising this code. The use of the code, based on the chemical composition instead of the trade names of the products tested, allows for a more comprehensive analysis of the results since disinfectants are generally marketed on a local or national rather than an international basis: the same formulation may be sold under a variety of names in different countries. Appendix II is a listing of the product trade names

together with those of their manufacturers or suppliers.

Test procedure for surface disinfection experiments

Using a Gilson (Mandel) variable volume pipettor and disposable plastic tips, each disk was contaminated with virus suspended in faeces and dried as already described. A 20 uL volume of the disinfectant under test, at the manufacturer's recommended dilution, was applied directly over the entire surface of each virus-contaminated disk. The control disks received the same amount of TPB instead. After a contact time of one min at room temperature (22±2°C), a 980 uL volume of TPB was added to each well containing the disks. This was done in order to stop the action of the disinfectant through its dilution (1/50).

Following elution of the virus from the disks, those reaction mixtures that were cytotoxic to MA-104 cells were passed through a column of Sephadex LH-20 as described below. EBSS was used as a diluent to prepare 3 serial ten-fold dilutions of the reaction mixture. These together with the undiluted sample were then plaque assayed. Figure 2 gives a summary of the main steps involved in the test procedure.

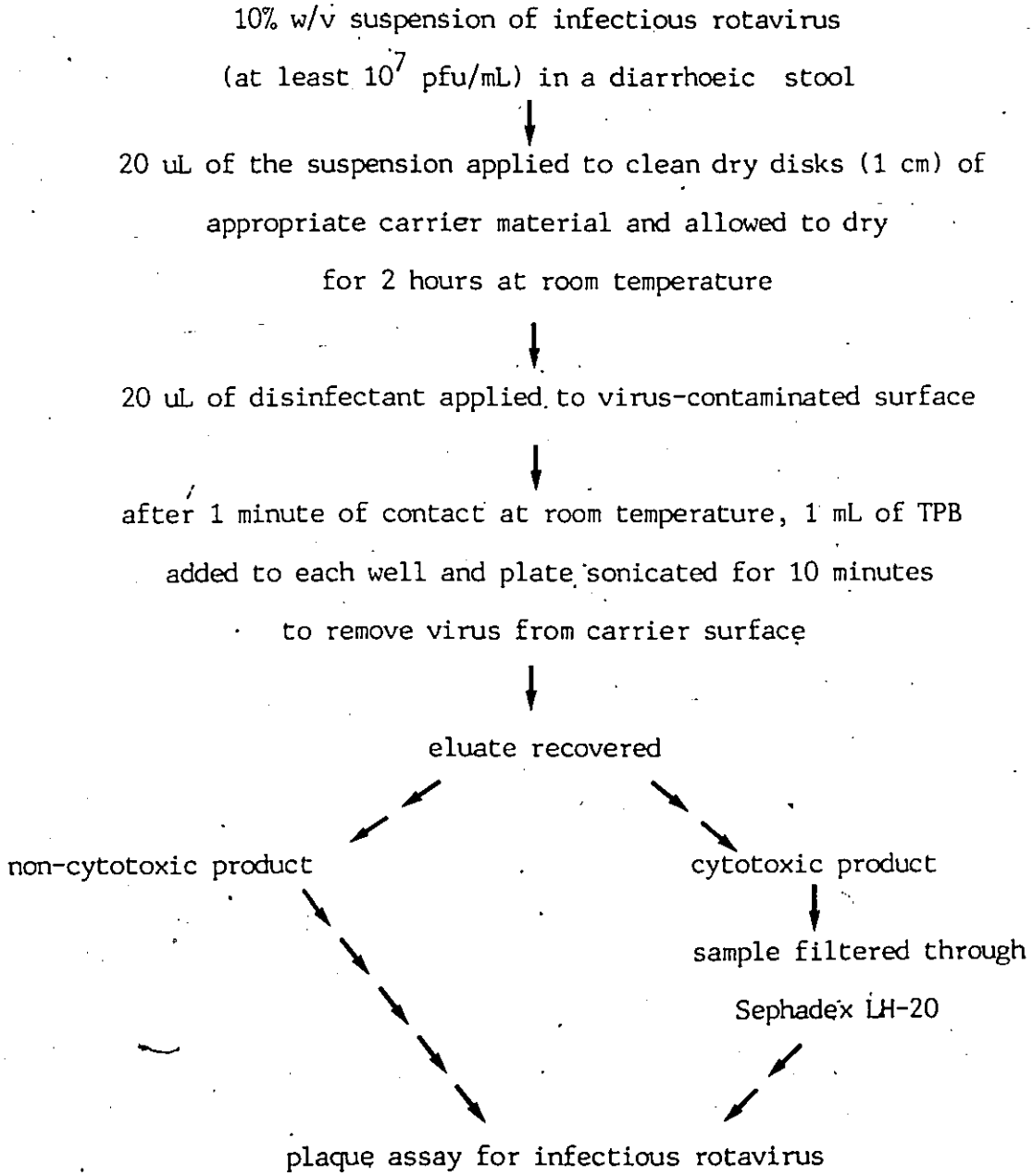
Whenever a disinfectant required dilution, unsterile tap water was used as a diluent. All tests in this study were repeated at least 6 times. A disinfectant was considered effective if it could bring about a 3 $\log_{10}$ (99.9%) or greater reduction in the plaque titre of the virus when compared to the control. For reasons to be described in the Results section, products found effective were further tested for their capacity to cause at least a 6 $\log_{10}$ reduction in virus titre.

Removal of cytotoxicity

Some disinfectants proved to be cytotoxic for MA-104 cells even after the initial 1:500 dilution of the virus-disinfectant mixture. The gel filtration technique of Blackwell & Chen (1970) was used to remove toxic

FIGURE 2.

DETERMINATION OF THE ROTAVIRUCIDAL ACTIVITY OF CHEMICAL DISINFECTANTS AND ANTISEPTICS BY THE CARRIER TEST



components. A 7.0 mL Centriflo plastic cone (Amicon, Lexington, MA, U.S.A.), with the tip plugged lightly with cotton, was used for holding pre-swollen beads of Sephadex LH-20 (Pharmacia, Uppsala, Sweden). The cone was then placed in a sterile plastic centrifuge tube and centrifuged at 1,000 rpm for 10 min at 4°C to pack the beads. The material to be treated was added to the top of the gel in the cone and centrifuged again as above. The filtrate collected in the centrifuge tube was removed and plaque assayed.

RESULTS

A) Rotavirus Survival

Selection of a suitable virus eluent

In preliminary experiments using ^{14}C-labelled HRV, a number of inorganic and proteinaceous solutions were tested for their capacity to elute the virus from the disks (Table 4). TPB was selected as the eluent because it could consistently recover 90-100% of the virus added to the disks of non-porous surfaces. Although its capacity for virus recovery from the porous materials was somewhat lower (70-93%); none of the other eluents tested, however, could perform any better. TPB was also shown to be harmless to rotavirus infectivity (Ramia and Sattar, 1980), non-toxic to MA-104 cells and contributed to the neutralisation of disinfectant cytotoxicity in the disinfection experiments. Furthermore, the use of TPB as eluent was consistent with previous studies on rotavirus survival in water (Raphael et al. 1985) and in the aerosol state (Ijaz et al. 1985b) which enabled us to make direct comparisons of the capacity of this virus to survive under different environmental conditions. Therefore, in all subsequent experiments TPB was used to recover HRV from the contaminated disks.

Virus survival on non-porous materials held at 22±2°C

There was virtually no loss in HRV infectivity on the disks of the non-porous materials during the two hour period allowed for the initial drying of the virus suspension. However, as shown in Table 5, when the contaminated disks were held over a longer period at 22°C, RH was found to have a pronounced influence on virus survival. The virus survived equally well at the medium and low RH levels (Ki 0.046-0.072 and Ki 0.025-0.048, respectively) and under these conditions, approximately 10% of the virus remained infectious on the disks even after 10 days. On the other hand, at 22±2°C virus survival at the high RH was dramatically different (Ki 1.564-

Table 4

a) % RECOVERY OF ^{14}C-LABELLED HUMAN ROTAVIRUS SUSPENDED IN FAECAL MATTER FROM CONTAMINATED SURFACES BY VARIOUS ELUENTS

	Eluent					
Surface	TPB	EBSS	PBS	ARG/GLY	BSA	DDW
STAINLESS STEEL	94(4.6)	91(5.2)	92(5.8)	94(4.0)	94(4.0)	93(4.0)
ROUGH PLASTIC	91(5.2)	90(5.8)	93(5.8)	93(4.6)	91(3.5)	92(4.6)
SMOOTH PLASTIC	87(5.8)	92(6.9)	93(8.7)	92(4.0)	93(4.6)	90(10)
GLASS	95(4.0)	98(8.1)	95(6.4)	100(5.2)	95(5.2)	95(4.6)
CARD	71(2.8)	75(5.7)	75(2.8)	78(3.5)	73(2.8)	74(3.5)
CLOTH	86(14)	77(10)	83(15)	92(13)	78(16)	77(9.0)
DOLLAR BILL	89(20)	81(24)	92(17)	93(15)	84(13)	74(7.1)

b) % RECOVERY OF INFECTIOUS HUMAN ROTAVIRUS SUSPENDED IN FAECAL MATTER FROM FOUR DIFFERENT TYPES OF PAPER USING TRYPTOSE PHOSPHATE BROTH

BEDSIDE CHART	49.0(7.8)
LABORATORY REQUEST FORM	114.0(39.7)
BOND PAPER	50.0(5.7)
100% COTTON PAPER	42.1(12.4)

Figures in parenthesis are standard deviations of results from three experiments.

Table 5

INACTIVATION RATES (Ki) OF HUMAN ROTAVIRUS AT VARIOUS RELATIVE HUMIDITY LEVELS ON FOUR DIFFERENT TYPES OF NON-POROUS INANIMATE SURFACES HELD AT 22±2°C

Surface	Relative humidity (%) levels		
	25±5	50±5	85±5
STAINLESS STEEL	0.033	0.046	1.741
ROUGH PLASTIC	0.025	0.072	1.564
SMOOTH PLASTIC	0.031	0.072	1.868
GLASS	0.048	0.069	2.408

HRV suspended in a faecal suspension, was applied to 1 cm diameter disks, allowed to dried for 2 hours and sampled at 6 time intervals over 10 days. The loss of virus infectivity was calculated by regression analysis at each RH level and the Ki is expressed as loss of virus infectivity in $\log_{10}$ PFU/day.

2.408; $P < 0.001$), with >99.9% reduction in virus titre within 48 h. Since there was no significant difference in the rate and pattern of HRV inactivation on the four types of non-porous surfaces tested, only the results obtained with stainless steel were selected for a graphic representation (Figure 3).

Virus survival on stainless steel disks held at 4°C

To examine the effect of lower air temperature on HRV survival on non-porous surfaces, a series of experiments was conducted at 4°C using disks of stainless steel. The results of these tests, carried out at the medium RH level, showed that the capacity of HRV to survive on such materials was further enhanced; however, when compared to the results of the experiments at 22°C, this increase in virus survival at the low temperature was not statistically significant (Table 6). In contrast to this, the rate of virus inactivation ($Ki = 0.080$) at the high RH level and 4°C was significantly lower ($P < 0.001$) than at the same RH and 22°C. Furthermore, at 4°C, the observed difference between the rates of virus inactivation at mid and high RH levels was no longer statistically significant.

Virus survival on stainless steel disks held at 37°C

Experiments conducted at 37°C and the low RH level, to reflect conditions similar to that in some tropical countries, indicate that, although the rate of virus inactivation (Ki approximately 0.197) was higher than at 22°C ($Ki = 0.033$), HRV could survive on stainless steel disks for at least 6 days under these conditions.

Effect of faecal matter

Only minor differences in HRV survival were observed among the faecal samples used in this study. To determine if faecal material itself had an effect on HRV survival, experiments were performed under ambient conditions

Figure 3

THE EFFECT OF VARIOUS LEVELS OF RELATIVE HUMIDITY ON THE CAPACITY OF HUMAN ROTAVIRUS (Wa) TO SURVIVE ON DISKS OF STAINLESS STEEL HELD AT 22±2°C

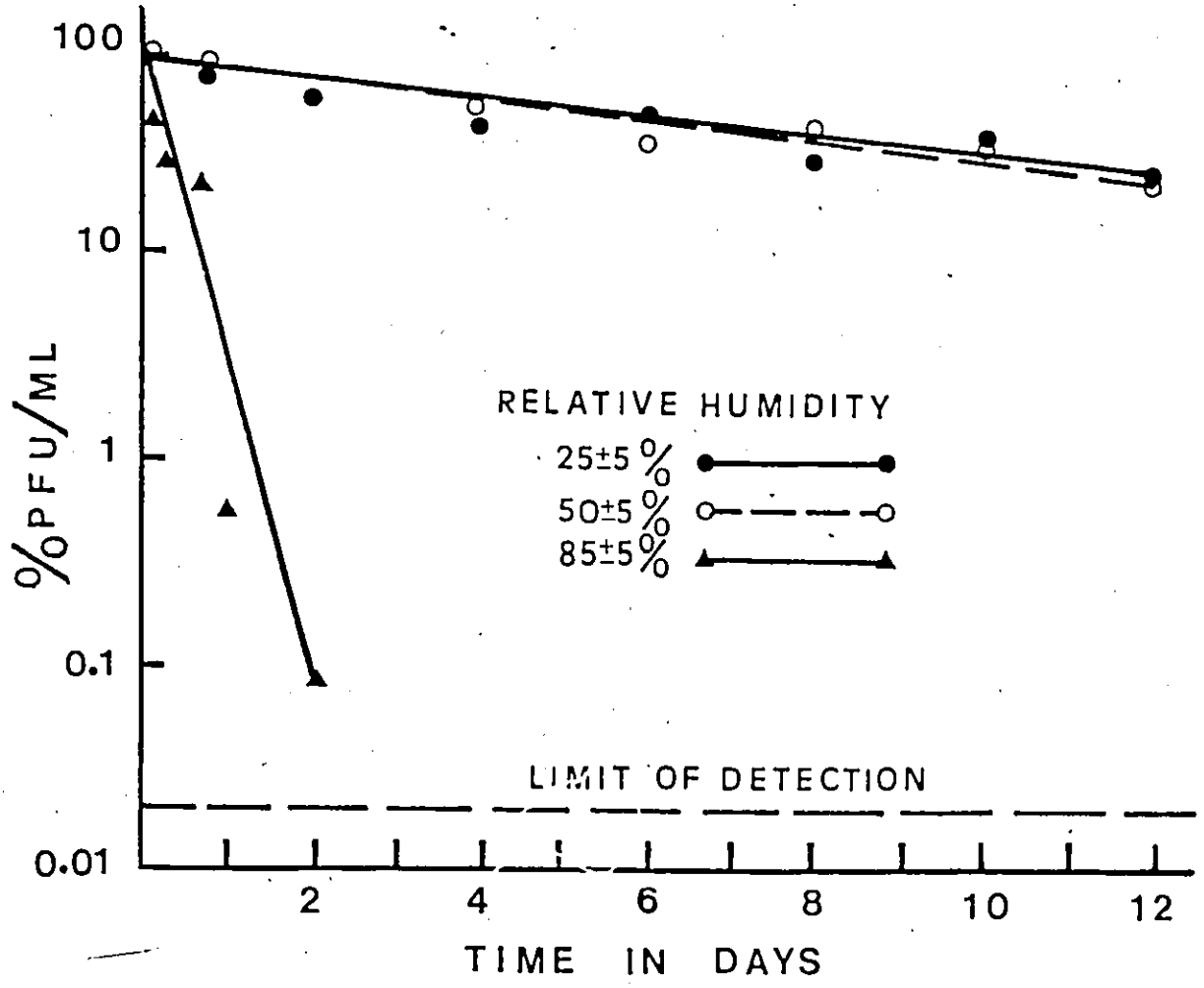


Table 6

INACTIVATION RATES (Ki) OF HUMAN ROTAVIRUS
AT VARIOUS RELATIVE HUMIDITY LEVELS
ON STAINLESS STEEL DISKS HELD
AT 22±2°C OR 4°C

Temperature	Relative humidity (%) levels		
	25±5	50±5	85±5
22±2°C	0.033	0.046	1.741
4.0°C	NT	0.0008	0.080

NT = not tested.

For procedure, see footnote of Table 5.

(50% RH, 22°C) with the virus suspended in TPB rather than faecal material. The rate of virus inactivation here was greater (Ki = 0.233) than that observed under identical conditions but with faeces (Ki = 0.046) as the virus suspending medium.

Effect of RH changes on virus survival

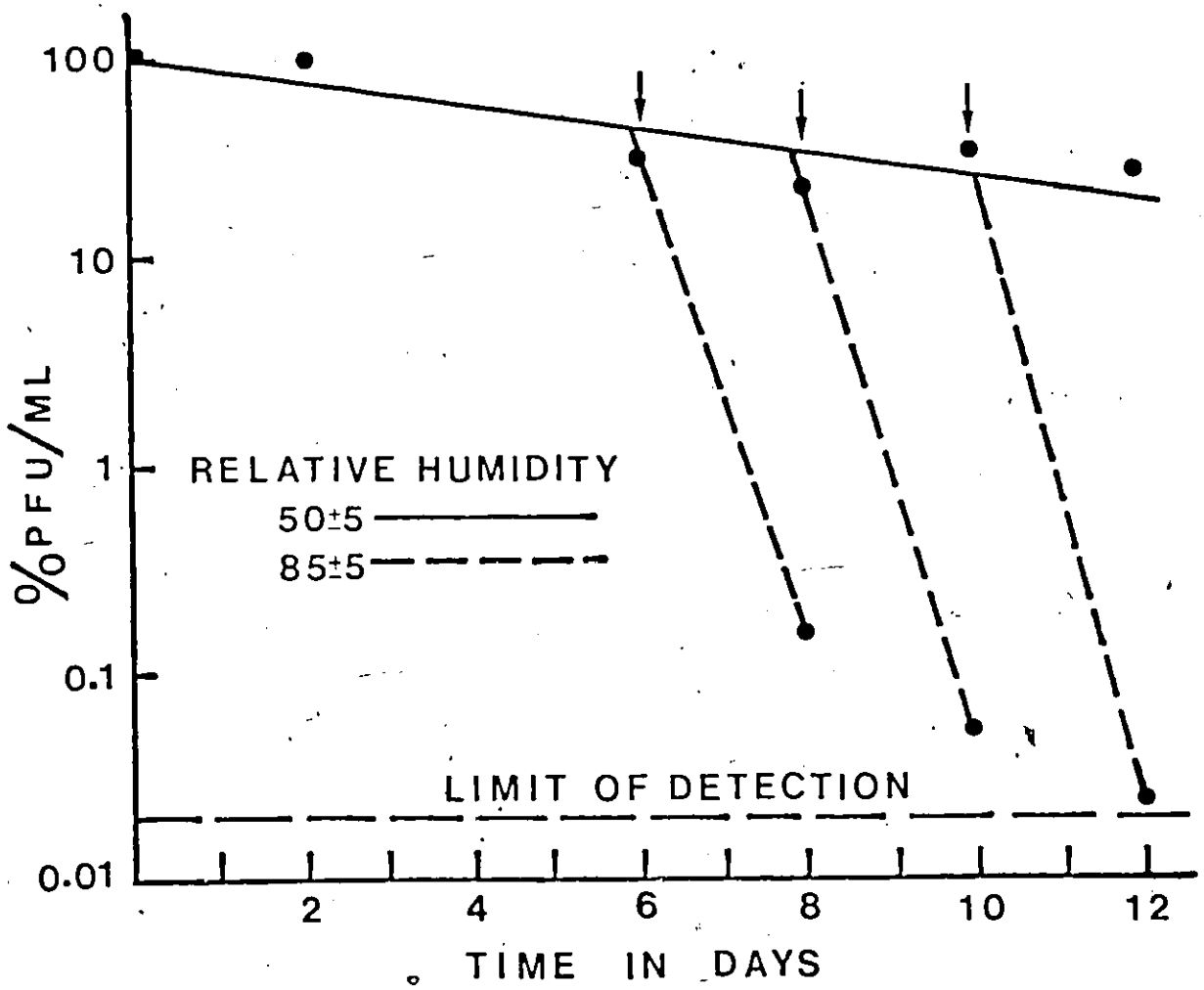
In many natural settings there are wide variations in RH levels during the course of a day. Figure 4 shows the results from experiments where virus-contaminated disks of stainless steel were shifted from mid to high RH. On days 6, 8 and 10, some disks held at mid-range RH were transferred to the high RH level, and kept there for a two-day period. Whereas the virus continued to survive well on those disks left at the mid-range, there was a 3-4 $\log_{10}$ reduction in the virus titre on the ones shifted to the high RH. No virus reactivation (data not presented in Figure 4) was observed when disks held at the high RH for two days were returned to mid-range RH for a further two-day period. Therefore, in this experimental set-up, virus inactivation occurring at the high RH was irreversible.

Virus survival on porous materials

Virus survival on the 7 types of porous materials tested was variable. Between 50-80% of infectious HRV particles survived on the four types of writing paper after the initial 2 h of drying. No infectious HRV could be recovered in any of the tests with experimentally-contaminated samples of poster card, even immediately after the drying period. This indicated that virus inactivation on this material was quite rapid. The anti-viral property of the card could not be eliminated by soaking it for 2 h in either TPB or 95% ethanol. The virus was not inactivated by the TPB obtained after the soaking of the card, indicating the non-water soluble nature of the anti-viral component(s) that may be present in this material. As has been mentioned earlier, TPB, when used as a virus eluent, could recover

Figure 4

INACTIVATION OF HUMAN ROTAVIRUS (Wa) ON STAINLESS STEEL DISKS WHEN RELATIVE HUMIDITY IS SHIFTED FROM THE MEDIUM TO THE HIGH LEVEL AT 22±2°C



between 70 and 95% of the radioactivity contained in HRV added to disks of such porous materials. Therefore, the drop in virus infectivity seen in these experiments was not due to the irreversible binding of infectious virus to the matrix of the card.

Infectious virus particles were occasionally recovered from experimentally-contaminated samples of currency paper, but the titres were too low and variable for accurate quantitation and statistical analysis.

The variability of HRV survival on the laboratory-contaminated pieces of cloth was such that, in some instances, no infectious virus could be recovered, whereas, at other times the inactivation rates were comparable to those of the non-porous surfaces tested under identical conditions. For example, in one set of experiments, infectious HRV could be recovered from them up to 2 days at 22°C, and up to 10 days at 4°C. When working with this material, the influence of high RH on virus survival at 22°C also appeared to be less marked than was observed for the non-porous surfaces. This wide variation in HRV survival on cloth could not be eliminated by prior washing of the disks.

B) Rotavirus Disinfection

The results of the carrier tests for the chemical disinfection of rotavirus-contaminated non-porous surfaces are summarised in Table 7. For comparison, the performance rating of each product or formulation in the suspension tests (Springthorpe et al., 1986) is also listed. An additional section of the table gives the results on those disinfectants that were not examined by the suspension test. It should, however, be noted here that the numerical identification of these products does not correspond to the number assigned to the same product in the suspension tests (Springthorpe et al. 1986).

TABLE 7

EFFICACY OF DISINFECTANT FORMULATIONS AGAINST HUMAN ROTAVIRUS IN THE CARRIER TEST

Disinfectant Formulation	Listed Composition	Dilution Tested	Recommended Use	Suspension Test Rating*	>99.9% loss in PFU
1	PHA 15.00% IAL 18.00% DBS 14.55% HCL 4.70% SUX 0.07%	1:128	toilet and urinal cleaner	A	NO
2	HCL 8.50% QAO 0.25%	undiluted	toilet bowl cleaner	A	YES
3	IAL 45.00% PRG 2.00% TRG 3.00% QAC 0.30%	undiluted	sanitize air and deodorise environmental surfaces	A	YES
4	QAE 0.05% QAL 0.05% EAL 19.68% NAM 2.50%	undiluted		A	YES
5	EAL 95.00% EAL 70.00%	undiluted undiluted	general disinfection of contaminated fluids and surfaces in laboratories	A A	NO NO
6	IAL 70.00%	undiluted		A	NO
7	CHG 1.50% CET 15.00%	1:30 diluted in EAL	topical and instrument disinfectant	A	YES
8	GLT 2.00%	undiluted	disinfection of instruments for gastrointestinal examinations	A	YES
9	CFT 67.00%	1:40	general purpose sanitizer for food and beverage handling equipment	A	YES
10	POV 10.00% (1% available I_2)	undiluted	all purpose skin disinfectant	A	YES

TABLE 7 (continued)

Disinfectant Formulation	Listed Composition		Dilution Tested	Recommended Use	Suspension Test Rating*	>99.9% loss in PFU
11	OPP BCP TPP	1.70% 2.50% 0.90%	1:80	disinfection in hospitals, clinics and surgical areas	A	NO
12	SBC SDS	0.50% 0.60%	undiluted	general domestic use, especially bathroom surfaces	A	YES
13	QAE QAL NAM	0.10% 0.10% 0.23%	undiluted	general purpose cleaner for all water washable surfaces	B	NO
14	QAC NAM TYT	1.60% 2.50% 1.50%	1:20	disinfection and cleaning of water washable inanimate surfaces	B	NO
15	OAA NAC	15.10% 2.73%	1:12	general purpose hard surface disinfectant	C	NO
16	QAE QAL EAL	0.09% 0.09% 19.68%	undiluted	disinfection of water washable inanimate surfaces	C	NO
17	SHC	6.00%	1:9	hard surface disinfectant	C	NO
18	NPE PHA	18.76% 15.96%	1:167	inanimate surfaces	C	NO
19	NPP PNP	8.74% 9.10%	1:100	general purpose germicide for industrial or institutional use on inanimate surfaces	C	NO
20	CHX IAL	4.80% 9.40%	1:40	topical and general disinfectant	C	NO
21	CDP NTR	0.50% 0.10%	undiluted	antibacterial hand-soap	C	NO
22	CHG CET	1.50% 15.00%	1:200	general purpose disinfectant	D	NO
Disinfectants and chemicals not tested in the suspension tests						
23	WAL	70.00%	undiluted		not tested	NO

TABLE 7 (continued)

Disinfectant Formulation	Listed Composition	Dilution Tested	Recommended Use	Suspension Test Rating*	>99.9% loss in PFU
24	PER 35.00%	1:100 1:1000	disinfection of instruments and hard surfaces	not tested "	YES YES
25	ACE 100.00%	undiluted	opthalmological instruments	"	NO
26	QAL 2.25% QAF 2.25%	1:64	cleaner/disinfectant for environmental surfaces	"	NO
27	HPO 30.00%	1:100	plastic surgical implants and contact lens	"	NO

* The disinfectants evaluated in the suspension test studies (Springthorpe et al. 1986) have been rated as follows:

A = highly effective; i.e. brings about a >3 $\log_{10}$ reduction in virus titre in the presence of high organic load (29.5g/l of TPB/l).

B = moderately effective; i.e. brings about a >3 $\log_{10}$ reduction in virus titre in the presence of low organic load (14.75g TPB/l) but not in high organic load (29.5g TPB/l) or 10% suspension of diarrhoeic faeces.

C = slightly effective; i.e. effective in the absence of added organic load but not when TPB or faecal matter is present.

D = ineffective; i.e. produces little or no reduction in virus titre even in the absence of added organic matter.

In initial experiments, the virucidal capacity of representative products was assessed in triplicate tests on each of the four different types of non-porous surfaces. Since no variation in their disinfection efficacy was observed among the surfaces, all further tests were carried out on the disks of stainless steel only.

Of the formulations rated A in the suspension test (Springthorpe et al. 1986) 8 of the 12 that were further evaluated by the carrier test were able to reduce the infectious HRV titre by >3 $\log_{10}$. Surprisingly, high concentrations (70% v/v) of alcohols on their own (#5, & #6), were ineffective in this regard. However, alcohols at concentrations >40% in combination with other active ingredients (#3, #4, #7), showed marked rotavirucidal activity. Product #1 which contained only 18% isopropanol was ineffective. The other formulations which were effective against HRV include 2% glutaraldehyde (#8), 2.5% chloramine T (#9), 10% povidone-iodine with 1% available iodine (#10), a phenolic compound containing an anionic surfactant (#12), our modification of a quarternary ammonium-based product (#4) and an acid (HCl) in combination with a quaternary ammonium compound (#2). One acid-containing product (#1) which was ineffective in disinfecting rotavirus-contaminated surfaces, contained a combination of acids (including HCl) at very low concentrations at its in-use dilution. The only other ineffective product in this category was a combination of 3 different phenolics with an in-use dilution of 1:80.

Neither of the disinfectants (#13 & #14) with a B rating in the suspension test was effective in the disinfection of rotavirus-contaminated surfaces. Formulations which received a C or D rating in suspension tests were further examined in the carrier test. Their inclusion in this study helped to ascertain the validity of the suspension test as a predictive tool. As can be seen from Table 7, all of these formulations were found to

be incapable of reducing the HRV titre by $3\log_{10}$ in the carrier test.

Five compounds or formulations, which had not been previously examined in the suspension test, were also tested for their ability to disinfect HRV-contaminated carriers. Methanol (#23) was tested as a 70% solution to see if it was any more effective than ethanol and isopropanol in the disinfection of HRV-contaminated surfaces; it was not. Peracetic acid has been reported to inactivate several enteric viruses found in sewage effluents (Harakeh, 1984). Sporkenbach, Wiegers & Dernick (1981) have also shown the efficacy of peracids and compounds which generate them in the disinfection of a variety of viruses in the suspension test. Although peracetic acid is unlikely to be widely used because of both its instability and its possible cocarcinogenic properties, the results we obtained with pure peracetic acid (#24), at either 0.1% or 1.0% show that it was able to reduce the HRV titre on virus contaminated surfaces by $> 3 \log_{10}$ and could, therefore, be an effective disinfectant against HRV at these concentrations.

Acetone (#25) was included here because it has been suggested as a suitable virucide for disinfecting ophthalmological instruments (Drews, 1977). However, the results we obtained using acetone against HRV dried onto surfaces suggest that it may not be adequate for the proposed use. Formulation #26 is another quaternary ammonium-based disinfectant intended for general disinfection of hard surfaces. It was found to be ineffective against HRV in the carrier test.

In view of the successful disinfection of HRV-contaminated carriers by peracetic acid, the action of hydrogen peroxide in the carrier test was also evaluated. Hydrogen peroxide at 0.3% (#27) was not effective. However, the use of hydrogen peroxide concentrations ranging from 3-6% has been

suggested for surface disinfection of surgical implants (Turner, 1983). Further tests with higher concentrations of this substance may, therefore, be required.

Because large numbers of HRV particles are usually found in the faeces of infected individuals, the validity of a 3 $\log_{10}$ reduction in virus titre for disinfectant efficacy is often questioned. Therefore, disinfectant formulations which in our system produced a >3 $\log_{10}$ reduction were further tested and were shown to produce a greater than 6 $\log_{10}$ reduction in infectivity of HRV in the carrier tests (Table 8). This strongly indicates that if a disinfectant could reduce the HRV titre by 3 $\log_{10}$, then it could be considered capable of bringing the virus titre to an undetectable level.

Table 8

CAPACITY OF CHEMICAL DISINFECTANTS TO INACTIVATE HUMAN ROTAVIRUS ON CONTAMINATED NON-POROUS SURFACES

DISINFECTANT FORMULATION # *	PRODUCT NAME & COMPOSITION	SUSPENSION TEST RATING	>3 LOG_{10} REDUCTION	>6 LOG_{10} REDUCTION
3	Air Sanitiser QUATERNARY AMMONIUM/ >40% ALCOHOL	A	YES	YES
12	Bathroom Fantastik PHENOL/SDS/ALKALI	A	YES	YES
8	Cidex GLUTARALDHYDE (2%)	A	YES	YES
16	Endbac II QUATERNARY AMMONIUM/ SMS (2.5%)	A	YES	YES
2	Lysol Toilet Bowl Cleaner QUATERNARY AMMONIUM/ ACID (HCL)	A	YES	YES
9	Multichlor CHLORAMINE T	A	YES	YES
10	Proviodine ORGANOIODINE (1% available I_2)	A	YES	YES
7	Savlon CHLOROHEXIDINE GLUCONATE, (1.5%) CETRIMIDE (15.0%) ETHANOL (70 %)	A	YES	YES
24	PERACETIC ACID	A	YES	YES

* As previously shown in Table 6.

DISCUSSION

A) Rotavirus Survival

If fomites and environmental surfaces are to play a role in the transmission of HRV infections, then the virus must be able to survive on them. Therefore, in this study the capacity of HRV to survive on a variety of materials usually found in institutional, commercial and domestic settings, was evaluated under different environmental conditions.

The data presented here demonstrates that HRV in faecal matter can survive for prolonged periods on several types of inanimate materials. The stability of the virus was influenced by environmental factors such as relative humidity, temperature and the type of surface contaminated.

The air temperatures selected here represented those in climate-controlled buildings (22°C), cold-storage conditions (4°C) and the ambient indoor air (37°C) in the tropics during certain months of the year. The low and high RH levels selected represent extremes in the indoor RH due to seasonal variations in many temperate and tropical regions. In most modern air-conditioned buildings, on the other hand, the RH is generally maintained at the mid-range (50%) throughout the year.

The surfaces at most risk of exposure to direct or indirect contamination with virus-containing matter include our selection of porous and non-porous surfaces. Because of the difficulty in obtaining appropriate disks of enamel and porcelain (materials commonly used for bathroom fixtures), these two types of surfaces could not be included in this study. However, based on the data reported here, it is considered highly unlikely that HRV survival on them would be any different from that on the other non-porous surfaces tested.

The poster card was included because such material is commonly used by

children in daycare centres and schools and is often carried by them into the home environment. This material was also similar to that used for keeping patient records in hospitals and nursing homes. The laboratory requisition and bedside temperature recording forms are always at high risk of contamination with rotavirus-containing matter. The other two types of paper are used mainly for writing purposes and subjected to much handling by personnel in high risk areas as already mentioned above. Paper currency was selected as a potential vehicle for rotaviruses because of its high incidence of being handled and because it frequently forms a link between the outside community and the institutional or domestic environment.

To closely simulate natural conditions of environmental contamination, the virus used was added to a suspension of faeces in normal saline. This dilution was necessary to make the stool samples less inhibitory for the added virus used for experimental contamination. Moreover, the particulate matter present in the undiluted faeces was also a source of interference in the accurate dilution and quantitation of the virus. As can be seen from the results presented in this study, the presence of diluted faecal matter in the virus suspension protected virus infectivity better than TPB. This suggests that indigenous virus may survive even better on fomites and environmental surfaces when it is contained in undiluted faecal matter.

In the only other comparable study, Moe and Shirley (1982) showed that a field strain of HRV could survive better on contaminated glass coverslips when the RH was kept either at the low (12-34%) or high (75-94%) level as compared to the medium level (51-59%). Even at the medium RH level, however, they found the virus capable of surviving for 9 days. The results of our study using a laboratory-adapted strain of HRV also showed that this virus can persist for protracted periods on a variety of surfaces.

On the other hand, our observation that HRV survived least well at high RH levels disagrees with their results on HRV survival on surfaces. The reasons for this disparity in the findings of the two studies cannot be pinpointed at this stage. One difference between the design of the two studies was the method of generating and maintaining the humidity level. In their experimental set-up, coverslips contaminated with rotavirus-positive faeces were placed in closed containers and the RH inside them was maintained with the help of saturated salt solutions. In our procedure, air at the desired RH level was circulated through the chamber containing the disks to give approximately 6 air changes per hour. Furthermore, in our study the virus-contaminated disks were allowed to dry before exposure to the various humidity levels tested, whereas it is not clear if the same procedure was adopted by the other investigators. In spite of these differences in experimental protocols, the possibility of variations in survival characteristics between HRV strains cannot be completely ignored. In support of our data, a similar relationship has been found between RH and the capacity of three different types of rotaviruses to survive in the airborne state (Sattar et al., 1984; Ijaz et al., 1985a). Our findings that shifts of virus-contaminated surfaces from mid to high RH level caused rapid and irreversible inactivation of the virus provide further evidence that high RH is detrimental to virus survival at ambient temperature.

The susceptibility of HRV to high RH levels demonstrated in this study immediately suggests the possibility of using RH modification in the control of rotavirus outbreaks in institutional settings. However, caution should be exercised in this regard, because strain differences often exist within and between outbreaks of rotaviral disease (Rowland, et al., 1984) and it is not known if different HRV strains exhibit different survival patterns with respect to RH. Furthermore, it is not unknown for more than

one virus outbreak to be occuring simultaneously (Meissner et al., 1984), and the viral agents involved may show varying susceptibilities to RH. Therefore, any use of RH modification in the control of rotavirus outbreaks should be carried out in conjunction with proper disinfection practices.

The factors influencing the variability of HRV survival on the cloth and papers tested in this study could not be conclusively determined. Chemical preservatives or surfactants that may be present in such porous materials (Sidwell, Dixon & McNeil, 1967; Sidwell et al., 1970), and which were not removed by the washing procedure, could have virucidal activity. It is also possible that such chemical residues (e.g. detergents and fabric softeners used in laundering) may not be uniformly distributed throughout the fabric and may account for the variations observed on the cloth. On the other hand, when HRV did survive on the cloth, the observed inactivation rate was comparable to that on the non-porous surfaces. Because the substances that are used in the production of paper vary from one manufacturer to another, no generalisations with respect to paper can be made from the results of this study.

The enhanced stability of HRV at 4°C observed in this study makes it reasonable to assume that there may be hazards from handling objects contaminated before cold storage.

Seasonal factors which predispose susceptible individuals to rotaviral gastroenteritis are not known. In temperate climates, however, there is a sharp increase in the number of cases of rotaviral infections in the general community in the winter season (Murphy, Albrey & Crewe, 1977; Cubitt & Holzel, 1980; Holzel et al., 1980; Noone & Banatvala, 1983; Keswick et al., 1983b). Therefore, it is conceivable that such cases entering day-care centres or hospitals could initiate institutional outbreaks of rotaviral

diarrhoea.

Studies on the epidemiology of rotaviral gastroenteritis in developing countries with tropical and semi-tropical climates also report seasonal variations but demonstrate no consistent pattern. While the majority show an increase in the number of such cases in the cool dry season or the hot wet season (Maiya et al., 1977; de Torres et al., 1978; Black et al., 1981; Stintzing et al., 1981; Paul & Erinle, 1983; Mata et al., 1983; Goh et al., 1985; Sitbon et al., 1985) others report a changing pattern of frequency within a single country from year to year (Hieber et al., 1978; Mathan, 1984). These studies suggest that epidemics of HRV diarrhoea are not entirely due to seasonal variations in environmental conditions. They also raise the possibilty that many different types of vehicles, acting singly or in various combinations, may play a role in the spread of this virus.

In the general community, food (Hara et al., 1978) and drinking water (Lyckė et al., 1978; Harris, Cohen & Lippy, 1983; Hung et al., 1984) have been incriminated as vehicles in some outbreaks of acute diarrhoea due to rotaviruses. However, institutions located in urban areas generally receive their drinking water from well-monitored municipal facilities, and their food from centralised kitchens. Therefore, it is considered unlikely that these vehicles would be important in outbreaks of rotavirus diarrhoea that occur in such settings.

Rotaviruses can survive well in the airborne state (Sattar et al., 1984; Ijaz, 1985), but the role of air as a vehicle in the direct spread of rotaviral infections remains to be shown. It is conceivable, however, that airborne infectious rotavirus particles could also lead to the indirect spread of rotaviral infections through the contamination of fomites and environmental surfaces in the immediate surroundings of individuals excreting the virus (Totterdell, Chrystie & Banatvala, 1976).

Outbreaks of rotaviral diarrhoea, particularly those associated with hospitals and nursing homes, can result in increased mortality and morbidity (Halvorsrud and Orstavik, 1978; Marrie et al., 1982 ; Yolken et al., 1982). For example, in a prospective study, rotaviruses were found to be an important cause of infectious gastroenteritis in patients with bone marrow transplants (Yolken et al., 1982); the mortality rate among such infected patients was 55% as compared to 13% in those who remained uninfected. Nosocomial outbreaks of rotaviral diarrhoea have also been shown to add greatly to the total cost of hospitalisation for each affected individual (Ryder et al., 1977).

Rotaviruses also represent a potential health threat in the work environment. Published data are available to show occurrence of diarrhoea among staff in day-care centres (Lemp et al., 1984). Such overworked staff caring for ill infants have been found not to wash their hands regularly and are often unaware of the importance of handwashing and other hygienic practices controlling person-to-person spread (Pickering et al., 1981). In the hospital setting, medical and nursing staff frequently contract the infection during nosocomial outbreaks of rotaviral gastroenteritis (Hildreth, Thomas & Ridgeway, 1981; Hoh, Presser & Wigand, 1983). The frequency of handwashing amongst such personnel has been reported to be only 41% of the time after contact with patients (Albert & Condie 1981). Moreover there is evidence to show that such infected adults can be a source of rotaviruses for infants and young children (Kim et al., 1977). Here it is also important to note that children who acquire rotaviral diarrhoea in day-care centres or schools can carry the virus to their families (Haug, Orstavik & Kveldstad, 1978; Pickering et al., 1981; Grimwood et al., 1983; Keswick et al., 1983b).

Although it is yet to be determined whether an individual would become infected by the quantity of HRV that may be transferred from handling a contaminated surface, these findings emphasise the potential role of inanimate surfaces and fomites as vehicles of rotaviral diarrhoea. It is anticipated that the findings reported here will be of help in developing a clearer understanding of why domestic and institutional outbreaks of rotaviral diarrhoea frequently occur. They should also be of assistance in the formulation of more effective strategies for the prevention and control of such outbreaks.

B) Rotavirus Disinfection

Despite the magnitude of the global problem with rotavirus infections, and the routine use of disinfection as a part of measures used to control and prevent the spread of the disease, very limited published information is available on the virucidal activity of disinfectants against human rotaviruses. The disinfectants tested against HRV include chlorine-based products, ozone and peracetic acid (Tan & Schnagl, 1983; Harakeh & Butler, 1984; Harakeh, 1984) Work recently completed in this laboratory has also evaluated over 70 different commercially available products against HRV (Springthorpe et al. 1986). Narang & Codd (1983) examined a variety of agents against HRV, but it is difficult to properly evaluate their results which were based solely on ultrastructural changes (viewed by electron microscopy) induced by disinfectant treatment of virus suspensions prepared from rotavirus positive stools.

The above mentioned and other reports on animal rotavirus disinfection (Tan and Schnagl, 1981; Snodgrass and Herring, 1977; Kurtz, Lee & Parsons, 1980; Brade, Schmidt & Gattert, 1981; Sattar et al., 1983) are based on studies using suspension tests only. Although these studies show rota-

viruses to be resistant to a variety of commonly used disinfectants, comparative analysis of such results, as mentioned before, to get a comprehensive picture is difficult due to the diversity in experimental protocols used by the different investigators.

Apart from the data generated in this study, there have been no published reports on the chemical disinfection of HRV-contaminated surfaces. Here it should be emphasised that, when a disinfectant is to be used for hard surfaces, as an instrument soak or for topical application, it is inevitably contaminated surfaces which must be disinfected. Therefore, the suspension test is only useful as a prescreening tool to determine which disinfectants may be effective when applied to such surfaces.

Environmental contamination by pathogenic viruses generally occurs in the presence of body secretions and excretions. The organic and inorganic matter present in such materials provides the virus protection, not only from the prevailing atmospheric conditions but also against the action of a variety of chemical disinfectants. In view of this, a product is regarded as a reliable virucide only if it can rapidly inactivate virus both in suspension and on a contaminated surface in the presence of organic and inorganic matter. In this study we used faecally suspended HRV in order to closely simulate the level of challenge that a disinfectant would have to meet in order to be effective under natural conditions.

Here it should be noted that in faeces, rotavirus particles are often found either as large aggregates (Narang & Codd, 1981) or as clumps embedded in tissue fragments (Holmes, 1979; Williams, 1985). This would be expected to afford the indigenous virus even greater protection against the action of chemical disinfectants some of which are also rendered ineffective in the presence of such interfering factors. Preliminary experiments with HRV (Wa), carried out in this laboratory have shown this to be par-

ticularly true of stools which appear to contain high levels of lipids. It is not known whether this is due to the greater inaccessibility of the virus or the enhanced neutralisation of the disinfectant.

Although it was planned to include field strains of HRV in this study, faecal samples with sufficiently high titres of infectious rotavirus could not be obtained. Those faecal samples that were made available to us from CHEO contained no more than 4×10^3 rotavirus infective units/mL of a 10% (w/v) suspension, whereas a minimum of 10^5 infective units/mL was needed to properly demonstrate a 99.9% reduction in the virus titre. However, the validity of the data with the laboratory-adapted strain of HRV is supported by similar results obtained from suspension tests using simian rotavirus SA-11 (Sattar et al., 1983; Raphael, Sattar and Springthorpe, 1983) and calf rotavirus (Sattar et al., unpublished data).

The carrier tests in this study were performed using clean disks of non-porous surfaces. In nature, however, such surfaces may be coated with dust, dirt and a variety of residues and this is also expected to interfere with the rotavirus inactivating capacity of chemical disinfectants.

Although the total number of products tested in this study was much lower than that in the suspension test (Sattar, 1985), a close examination of each class of the effective disinfectants suggests that their ability to successfully inactivate HRV is related to certain combinations and/or minimum concentrations of specific ingredients. For example, quaternary ammonium compounds in combination with alcohols at concentrations >40% (#'s 3 and 7) or HCl (# 2) showed high rotavirucidal activity. The effectiveness of formulation #4, our modification of an otherwise ineffective quaternary ammonium-based product (#16), further strengthens these observations. Hendley et al. (1978) also made similar observations in their work on the

disinfection of rhinoviruses on hands; they report substantial inactivation of these viruses on hands by combinations of hexachlorophene/ethanol and benzalkonium chloride/ethanol whereas these substances on their own are known to be only partially effective or totally ineffective against non-enveloped hydrophilic viruses.

In addition, a comparison of these results of HRV disinfection using the carrier test, with the data based on the suspension tests (Springthorpe et al. 1986) shows that disinfection of HRV-contaminated surfaces is more difficult to achieve than the inactivation of the virus in suspension. This agrees with the findings of previous studies using other types of viruses (Kirchoff, 1969; Klein & Deforest, 1983; Nakao et al., 1978).

Kirchoff (1969), working with Newcastle disease virus, on a variety of porous and non-porous surfaces, suggests that virus on surfaces may be more difficult to inactivate than virus in suspension, and that the type of surface may influence the result. In our study however, we found no difference in disinfection efficacy among the four types of hard surfaces we tested. Klein & Deforest (1983) & Slobodenyuk et al. (1970) also suggest that higher concentrations of some germicides may be necessary for the inactivation of virus on surfaces. Nakao et al. (1978) observed that, transmissible gastroenteritis virus of pigs was 10 times as resistant to disinfection when it was dried onto a wooden surface than when in suspension. The antiviral activity of an alcoholic hand disinfectant on a variety of viruses both in suspension and on hands and fingertips, was examined by Schurmann & Eggers (1983). They also observed that the suspension test predicted a greater effectiveness of the disinfectant than was subsequently found in their tests on hands.

The results reported here also demonstrate that if a formulation was

ineffective in the suspension test, it was likewise incapable of disinfecting HRV-contaminated surfaces. However, as mentioned above, formulations that could effectively inactivate the virus in suspension, did not always do so on contaminated surfaces. This indicates that the suspension test, which is both quicker and simpler to perform than the carrier test, may be used only for the preliminary screening of potential virucides and not as a substitute for the carrier test. Therefore, formulations which are to be recommended for surface disinfection, particularly for general purpose use, should always be further evaluated on carriers.

Depending on the application, the contact time between virus and disinfectant can vary from a few seconds, in the case of hand washing and routine general purpose use, to several hours in the case of instrument soaks. However, it is generally agreed that in order to declare a disinfectant as efficient it should be able to produce a pronounced virucidal effect after a minimal contact time. The selection of a contact time of 1 minute not only afforded us a reproducible time interval, but also enabled us to obtain a more realistic picture of the usual practices of routine in-use surface disinfection. Furthermore, previous work in this laboratory has shown that, in the suspension test, disinfectants (with the exception of formaldehyde) that were not effective within the 1 minute contact time were not significantly more effective after 30 minutes of virus contact (Sattar et al., 1983). We believe that the use of a contact time of one minute allowed us to identify truly reliable products among those which show only marginal efficacy.

Our results have clearly shown that a surprisingly large proportion of chemical disinfectants and antiseptics commonly used in the institutional, professional and domestic environment are unsatisfactory for the proper

disinfection of rotavirus-contaminated materials. Available evidence strongly incriminates improperly disinfected fomites in the spread of rotaviral gastroenteritis (Ryder et al., 1977; Halvorsrud & Örstavik, 1980; Rocchi et al., 1981; McNulty & Logan, 1983). Therefore, because of the highly contagious nature of rotaviruses and the large volume of copious watery stools that can result from such infections, it is reasonable to assume that reliance on products which have little or no capacity for proper disinfection of HRV on surfaces may contribute to rotavirus transmission. Furthermore, improperly decontaminated objects and surfaces may pose a significant health hazard to persons handling them, while unaware of their contaminated state. Therefore, wherever possible, efforts should be made to select those products which have been shown to be effective in the inactivation of rotaviruses as well as other pathogenic viruses and bacteria. It is unreasonable to expect a single product to be suitable for use on surfaces, instruments as well as on hands, but those products which could inactivate HRV by a >6 $\log_{10}$ on our carriers represent formulations that could be suitable for these categories of use. Of those products deemed effective, two are favoured for topical use (#7 & #10), one for instrument soaks (#8) and the remaining six are recommended for general use on hard surfaces.

It is anticipated that the findings of this investigation will be of assistance in the proper selection of disinfectants and antiseptics for the prevention and control of outbreaks of rotaviral diarrhoea.

CONCLUSIONS AND AREAS FOR FURTHER RESEARCH

These investigations were aimed at elucidating the potential role of environmental surfaces and fomites in the transmission of rotaviral infections. The capacity of many commonly used chemical disinfectants was also evaluated in order to determine whether they can be utilised efficiently as part of measures instituted to control and prevent rotavirus spread by surface contamination. In the absence of standard tests for such studies, simple methods for virus recovery and disinfection on surfaces were developed.

The results obtained after two years of experimental work show that the main objectives of the study were realised. Therefore, the conclusions reached can be summarised as follows:

Our modification of the 'rinse' method for the recovery of HRV from surfaces is simple and highly efficient for hard non-porous surfaces, but recovery from porous materials, although successful, needs further study. In addition, our use of labelled virus provided a means by which we could accurately determine and monitor the efficiency of virus elution from the surfaces. In conjuction with the plaque assay, it enabled us to differentiate between the amount of virus inactivated and that irreversibly bound to the surface.

HRV was found to be capable of surviving for prolonged periods on all non-porous surfaces tested. Although its survival on the porous materials was somewhat variable, it could remain infectious on some of them for at least a few hours. Hence, such contaminated surfaces could be important vehicles of spread of HRV infections.

Environmental factors such as relative humidity and temperature both were found to have strong influence on HRV survival on surfaces. At the high RH level HRV was rapidly inactivated at room temperature but this effect was negated to a large degree when the air temperature was lowered

to 4°C. The mechanism(s) by which this inactivation of virus occurs remains largely unexplained. Therefore, further study is needed to understand the physicochemical changes that could account for the loss of virus infectivity under these environmental conditions. The relatively rapid inactivation of human rotaviruses at the high RH level suggests that humidification of the atmosphere in a room suspected of being contaminated with rotaviruses, may represent a simple and safe means of controlling and preventing outbreaks of due to these viruses. Further work is, however, required to test the feasibility of such a procedure under actual field conditions, particularly in hospitals.

Rotavirus survival on surfaces held at a higher air temperature (30-37°C) at different relative humidity levels also needs further study in order to broadly simulate the various environmental conditions found in many tropical and semi-tropical areas of the world.

A simple, effective and reliable protocol for testing chemical disinfection of rotavirus-contaminated surfaces was also developed. This procedure can be adapted for use in studies with other viruses.

The finding that not all disinfectant products which were effective in the suspension tests (Springthorpe et al. 1986), were able to produce the same effect on surfaces emphasises the unreliability of that test as the standard for disinfectant efficacy. The observation that no product that was ineffective in suspension tests could inactivate HRV on the carriers according to our criteria, clearly shows that the suspension test is a useful prescreening tool only.

The results of this study also point to the need for similar investigations on rotavirus survival on hands and their possible role in the spread of rotaviral infections.

Appendix I

ABBREVIATIONS FOR LISTED INGREDIENTS IN DISINFECTANT FORMULATIONS

ACE acetone

BCP o-benzyl-chlorophenol

BPP butoxypolypropoxypolyethoxyethanol-iodine complex

CDP 2,4,4',-trichloro-2'-hydroxydiphenyl ether (or 5-chloro-2(2,4-dichlorophenoxyl) phenol)

CET cetrimide

CHA chlorhexidine acetate

CHG chlorhexidine gluconate

CFT chloramine-T

CHX 4-chloro-3,5-xylenol

CIT sodium citrate

DBS dodecylbenzenesulphonic acid

EAL ethyl alcohol

GLT glutaraldehyde

HCL hydrochloric acid

HPO hydrogen peroxide

IAL 2-propanol

LAC lactic acid

NAC sodium chlorite

NAM sodium metasilicate

NPE nonylphenoxypolyethyleneethanol-iodine complex

NPP nonylphenoxypolyethoxyethanol-iodine

NTR nitrilotriacetic acid (as sodium salt)

OAA organic acid activator

OPP o-phenylphenol

PER peracetic acid

PDG polymeric diguanide hydrochloride

PHA phosphoric acid

PNP polyethoxypolypropoxypolyethoxyethanol-iodine complex

POV povidone-iodine complex

PRG propylene glycol

SBC sodium o-benzyl-p-chlorophenate

SDS sodium dodecylbenzenesulphonate

SHC sodium hypochlorite

SHO sodium hydroxide

SLS sodium dodecyl sulphate

SUX sulphuric acid

TPP p-tertiary amyl phenol

TRG triethylene glycol

TYT tetrasodium ethylenediaminetetraacetate

WAL methyl alcohol

QUATERNARY AMMONIUM COMPOUNDS are given below with the substituents on the quaternary nitrogen shown as R (1-4)

	R1				R2	R3	R4
	C12%	C14%	C16%	C18%			
QAC	40	50	10	-	methyl	methyl	benzyl
QAE	50	30	17	3	"	"	ethylbenzyl
QAF	68	32	-	-	"	"	"
QAI	50	30	17	3	"	"	benzyl
QAK	100% C10				C10	"	methyl
QAL	5	60	30	5	methyl	methyl	benzyl
QAO	isobutylphenoxyethoxyethyl				as R1	"	methyl

Appendix II

DISINFECTANTS AND ANTISEPTICS EVALUATED IN THIS STUDY. AGAINST ROTAVIRUS

FORMULATION NUMBER	COMMERCIAL NAME	MANUFACTURER
1	D'GERM	Sanfax Industries, Ltd.
2	LYSOL TOILET BOWL CLEANER	Sterling Drug.
3	AIR SANITIZER	Brentdale chemicals.
4	(Product # 16) + SODIUM METASILICATE	Fisher Scientific.
5	ETHANOL	Fisher Scientific.
6	ISOPROPANOL	Fisher Scientific.
7 & 22	SAVLON	Ayerst, Montreal.
8	CIDEX	Arbrook Ltd.
9	MULTICHLOR	Diversey (Canada) Ltd.
10	PROVIODINE	Rougier Inc.
11	TRIPLE PHENOLIC GERMICIDAL DETERGENT	Brentdale Chemicals.
12	BATHROOM FANTASTIK	Texize Canada Ltd.
13	BON AMI	S.C.Johnson & Son Ltd.
14	FORWARD	S.C.Johnson & Son Ltd.
15	ALCIDE LD	Alcide Corp. Wesport, Connec.
16	ENDBAC II	S.C.Johnson & Son Ltd.
17	JAVEX	Bristol-Myers.
18	SHUR-GAIN GERM KILL	Canada Packers Ltd.
19	WESCODYNE	West Chemical Products.

Appendix II (cont.)

20	DETTOL	Reckitt & Colman Overseas Ltd.
21	TRICLOSAN HAND SOAP	G.H.Wood.
23	METHANOL	Fisher Scientific.
24	PERACETIC ACID	Aldrich Chemicals.
25	ACETONE	Fisher Scientific.
26	PSP-QUAT	Professional Sanitation Products Ltd.
27	HYDROGEN PEROXIDE	Fisher Scientific.

APPENDIX III

Linear regression concerns the fitting of a straight line or curve to a scatter of experimentally observed data points, in order to conduct objective analysis of the data. The method of least squares was used to obtain a mathematically derived best-fitting line, about which the sum of the squares of the deviations of the observed points is a minimum. From this line one can obtain the slope (B) and the correlation coefficient (R).

In order to make inference from this least-squares regression line, it is necessary to determine the standard errors of the slope of this line (SE_B). Using composite data of at least three experiments. The mean, variance and standard deviation at each time interval, for each set of experimental conditions was calculated as follows;

$$\text{Mean} = \bar{y} = \Sigma y_i / n$$

n = number of observations.

$$\text{Variance} = V_{(y)} = \frac{\Sigma (y_i - \bar{y})^2}{(n-1)}$$

y = the ordinate value at each time interval.

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

The mean of the variance is calculated and used to compute the standard error (S_E) of the slope (B) of the regression line.

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

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

$S_{(y)}$ - is the mean variance of y.

B - is the slope of the line.

R - is the correlation coefficient.

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

The effect of a) surface type; b) relative humidity and c) temperature on virus inactivation with respect to time was then examined.

In order to perform tests of significance or to obtain confidence limits with regard to regression for any two of these parameters, the standard error of the B values of the two slopes is then directly compared in the "t"-test (two-tailed).

Where $$t = \frac{B_1 - B_2}{\sqrt{SE_{(B1)}^2 + SE_{(B2)}^2}}$$ with degrees of freedom $= (n_1 + n_2 - (4)$

EXAMPLES:

1) Virus recovery (% PFU/mL) from survival studies on stainless-steel disks held at 22±2°C and 50% level of relative humidity

Time (days)	(Experiment Number) % PFU/mL				
	(1	2	3	4	5)
0	100	100	100	100	100
2	34.1	149.3	55.5	47.0	-
4	21.4	53.7	83.9	-	127.3
6	10.9	14.6	33.9	28.0	77.3
8	26.4	79.1	87.5	17.0	38.6
10	15.5	28.4	98.2	19.0	54.5
12	-	-	-	21.0	30.0

2) Virus recovery (% PFU/mL) from survival studies on stainless steel disks held at 22±2°C and 85% level of relative humidity

Time (days)	(Experiment Number) % PFU/mL				
	(1	2	3	4	5)
0	100	100	100	100	100
0.08	-	-	60.6	28.3	70.9
0.16	-	-	20.91	63.3	16.4
0.75	-	-	-	20.0	34.5
1.00	0.29	0.11	0.30	1.7	0.00
2.0	0.00	0.00	0.00	0.09	0.00

(-) Not measured.

For graphical representation, $\log_{10}$ of y values was used, therefore, it is necessary to use the $\log_{10}$ y in the statistical computations.

Summary of statistical analysis for results of survival studies of HRV on stainless steel disks held at 22±2°C under different levels of relative humidity.

Humidity level	R	B	n	$V_{(y)}$	SE
25±5%	0.417	0.0326	14	0.0962	0.0814
50±5%	0.539	0.0459	30	0.0745	0.0434
85±5%	0.891	1.7412	17	0.0832	0.0338

SAMPLE CALCULATION USING DATA GIVEN ABOVE:

The t-test (two-tailed), comparing survival of HRV on stainless steel at 85% (B_1) and 50% (B_2) relative humidities.

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

$$= \frac{1.7412 - 0.0459}{\sqrt{(0.0338)^2 + (0.0434)^2}}$$

$$= \frac{1.6953}{\sqrt{0.0011424 + 0.0018835}}$$

$$= \frac{1.6953}{0.0550081}$$

$$= 30.376$$

Degrees of freedom
$= n_1 + n_2 - 4$
$= 17 + 30 - 4$
$= 43$

Therefore $P < 0.001$

REFERENCES

Albert, R.K. & Condie, F. (1981). Handwashing patterns in medical intensive-care units. New England Journal of Medicine 304, 1465-1466.

Arnow, P. M., Hierholzer, J. C., Higbee, J. & Harris, D. H. (1977). Acute hemorrhagic conjunctivitis: a mixed virus outbreak among Vietnamese refugees on Guam. American Journal of Epidemiology 105, 68-74.

Bean, B., Moore, B. M., Sterner, B., Petersen, L. R., Gerding, D. N. & Balfour, H. H. Jr. (1982). Survival of influenza viruses on Environmental Surfaces. Journal of infectious diseases 146, 47-51.

Bishop, R. F., Davidson, G. P., Holmes, I. H. & Ruck, B. J. (1973). Virus particles in the epithelial cells of duodenal mucosa from children with acute nonbacterial gastroenteritis. Lancet 2, 1281-1283.

Black, R. E., Huq, I., Merson, M. M., Alim, A. R. M. A. & Ynus MD. (1981). Incidence and severity of rotavirus and Escherichia coli diarrhoea in rural Bangladesh. Lancet 1, 141-143.

Blackwell, J. H. & Chen, J. H. S. (1970). Effects of various germicidal chemicals on H.Ep.2 cell culture and herpes simplex virus. Journal of the Association of Official Analytical Chemists 53, 1229-1236.

Bond, W. W., Favero, M.S., Petersen, N.J., Gravelle, C. R., Ebert, J. W. & Maynard, J. E. (1981) Survival of hepatitis B virus after drying and storage for one week. Lancet 1, 550-551.

Bond, W.W., Favero, M.S., Petersen N.J. & Ebert, W.J. (1983). Inactivation of hepatitis B by intermediate-to-high-level disinfectant chemicals. Journal of Clinical Microbiology 18, 535-538.

Bond, W. W., Petersen, N. J. & Favero, M. S. (1977). Viral Hepatitis B: Aspects of environmental control. Health and Laboratory Science 14, 235-252.

Bond, W. W. (1984). Survival of hepatitis B virus in the environment. Journal of the American Medical Association 251, 397-398.

Borick, P. M., Dondershire, F. H. & Chandler V. L. (1964). Alkalinized glutaraldehyde, a new antimicrobial agent. Journal of Pharmaceutical Science 53, 1273-1275.

Brade, L., Schmidt, W. A. K. & Gattert, I. (1981). Relative effectiveness of disinfectants against rotavirus. Zentralblatt fur Bakteriologie und Hygiene (Abteilung I. Originale), B, 174, 151-159.

Brandt, C. D., Kim, H. W., Rodriguez, W. J., Arrobio, J. O., Jeffries B. C. & Parrott, R. H. (1982). Rotavirus gastroenteritis and weather. Journal of Clinical Microbiology 16, 478-482.

Bridger, J. C. & Woode, G. N. (1976). Characterisation of two particle types of calf rotavirus. Journal of General Virology 31, 245-250.

Brown, T. T. Jr. (1981). Laboratory evaluation of selected disinfectants as virucidal agents against porcine parvovirus, pseudorabies virus and transmissable gastroenteritis virus. American Journal of Veterinary Research 42, 1033-1036.

Bryden, A. S, Davies, H. A., Hadley, R. E., Flewett, T. H., Morris, C. A. & Oliver, P. (1974). Rotavirus in the Westmidlands during 1974. Lancet 2, 241-243.

Buckland, F. E. & Tyrrell, D. A. (1962). Loss of infectivity on drying various viruses. Nature 195, 1063-1064.

Carlson, J. A. K., Middleton, P. J., Szymanski, M., Huber, J., Petric, M. (1978). Fatal rotavirus gastroenteritis. An analysis of 21 cases. American Journal of Diseases in Childhood 132, 477 (1978).

Champsaur, H., Questiaux, E., Prevot, J., Henry-Amar, D., Goldszmidt, D., Bourjouane, M. & Bach, C. (1984a). Rotavirus carriage, asymptomatic infection and disease in the first two years of life. Journal of Infectious Diseases 149, 667-674.

Champsaur, H., Henry-Amar, M., Goldazmidt, D., Prevot, J., Bourjouane, M., Questiaux, E. & Bach, C. (1984b). Serological response to rotavirus and virus shedding: a prospective study. Journal of Infectious Diseases 149, 675-682

Chen, J. H. S. & Koski, B. S. (1983). Methods of testing virucides. In Disinfection, Sterilization and Preservation. Block, S.S. (Ed.) Lea & Febiger, Philadelphia, 1983. pp. 981-997.

Chen, J.H.S. & Phebus, D.E. (1978). The influence of organic load on the efficacy of virucide against Influenza virus A2. Paper presented at ASTM Antiviral Agents Group Philadelphia-Meeting (March 16).

Chrystie, I. L., Totterdell, B., Baker, M. J., Scopes, J. W. & Banatvala, J. E. (1975). Rotavirus infections in a maternity unit. Lancet 2,79.

Cliver, D. O. & Kostenbader K. D. Jr. (1984). Disinfection of virus on hands for prevention of food-borne disease. International Journal of Food Microbiology 1, 75-87.

Cohen, J., Laporte, J., Charpilienne, A. & Scherrer, R. (1979). Activation of rotavirus RNA polymerase by calcium chelation. Archives of Virology 60, 177-186.

Cubitt, W. D. & Holzel H. (1980). Hospital acquired rotavirus infection in adults. Journal of Hospital Infection 1, 327-331.

Cukor, G & Blacklow, N. R. (1984). Human viral gastroenteritis. Microbiological Reviews 48, 157-179.

D'Alessio, D. J., Meschievtz, C. K., Petersen, J. A., Dick, C. R. & Dick, E. C. (1984). Short-duration exposure and the transmission of rhinoviral colds. The Journal of Infectious Diseases 150, 189-194.

D'Angelo, L. J., Hierholzer, J. C., Holman, R. C. & Smith, D. J. (1981). Epidemic keratoconjunctivitis caused by adenovirus type 8: epidemiologic and laboratory aspects of a large outbreak. American Journal of Epidemiology 113, 44-49.

Davidson, G. P., Bishop, R. F., Towley, R. R. W., Holmes, I. H. & Ruck, B. J. (1975). Importance of a new virus in acute sporadic enteritis in children. Lancet 1, 242-245.

de Torres, B.V., de Ilja, R. M. & Esparaza, J. (1978). Epidemiological aspects of rotavirus infection in hospitalised Venezuelan children with gastroenteritis. American Journal of Tropical Medicine and Hygiene 27, 567-572.

Dixon, G. J., Sidwell, R.W. & McNeil, E. (1966). Quantitative studies on fabrics as disseminators of viruses. II. Persistence of poliomyelitis virus on cotton and wool fabrics. Applied Microbiology 14, 183-188.

Downie, A. W., Meiklejohn, M., St. Vincent, L., Rao, A. R., Babu, B. V. S. & Kempe, C. H. (1965). The recovery of smallpox from patients and their environment in a smallpox hospital. Bulletin of the World Health Organisation 33, 615-622.

Drewes, R. C. (1977). Acetone sterilization in ophthalmic surgery. Annals of Ophthalmology 9, 781-748.

Drulak, M., Wallbank, A. M., Lebtag, I., Werboski, L. & Poffenroth, L. (1978a). The relative effectiveness of commonly used disinfectants in inactivation of coxsackievirus B5. Journal of Hygiene 81, 389-397.

Drulak, M., Wallbank, A.M. & Lebtag, I. (1978b). The relative effectiveness of commonly used disinfectants in inactivation of echovirus 11. Journal of Hygiene 81, 77-78.

Echeverria, P., Burke, D. S., Blacklow, N. R., Cukor, G., Charoenkul, C., & Yanggratoke, S. (1983). Age specific antibody to rotavirus, Escherichia coli heat-labile enterotoxin, Norwalk virus, and hepatitis A virus in a rural community in Thailand. Journal of Clinical Microbiology 17 923-925.

Edward, D. G. (1941). Resistance of influenza virus to drying and its demonstration on dust. Lancet 241,. 664-666.

Elias, M. M. (1977). Separation of two particle types of human rotavirus. Journal of General Virology 37, 191-194.

England, B. L. (1982). Detection of viruses on fomites. In Methods in Environmental Virology. Gerba, C.P. & Goyal, S.M. (Eds.) Microbiology series Vol.7, Ch. 9, pp.179-220.

Estes, M. K., Palmer, E. L. & Obijeski, J. F. (1983). Rotaviruses: A review. In Current Topics in Microbiology & Immunology 105, 123-184.

Evans, A. S. (1982). Epidemiological concepts and Methods. In Viral Infections of Humans: Epidemiology and Control. Evans, A.S. (2nd Ed.) 1982 Plenum Publishing Corporation, New York, NY. (2nd. ed) 1982 Plenum Publishing Corporation, New york, NY.

Evans, D. H., Stuart, P. & Roberts, D. H. (1977). Disinfection of animal viruses. British Veterinary Journal 133, 356-359.

Everall, P. H., Morris, C. A., Oliver, P. R. & Becker, J. F. (1982). Problems in the disinfection of Class 1 microbiology safety cabinets. Journal of Clinical Pathology 35, 698-705.

Favero, M. S., Bond, W. W., Petersen, N. J., Berquist R. K. & Maynard, E.

J. (1974). Detection methods for study of the stability of hepatitis B antigen on surfaces. Journal of Infectious Diseases 129, 210-212.

Fenner, F. J. & White, D. O. (1970). Medical Virology, Ch.10. pp. 182-201. Academic Press, New York.

Flewett T. H. (1983). Rotaviruses in the home and hospital nursery. British Medical Journal 287, 568-569.

Flewett, T. H., Bryden, A.S., Davies, H., Woode, G.N., Bridger, J. C. & Derrick, J. M. (1974). Relation between viruses from acute gastroenteritis of children and newborn calves. Lancet 2, 61.

Foster, O., Adjukiewicz, A., Ryder, R. & Whittle, H. (1984). Hepatitis B virus transmission in West Africa: A Role for tropical ulcers. Lancet 1, 576-577.

Foster, S. O., Palmer, E., L., Gary Jr., G. W., Martin, M. L., Herrman, K. L., Beasley, P. & Sampson, J. (1980). Gastroenteritis due to rotavieus in an isolated Pacific island group: an epidemic of 3,439 cases. Journal of Infectious Diseases 141, 32-39.

Freij, L., Sterky, G., Wadstrom T. & Wall, S. (1978). Child health and diarrhoeal disease in relation to water supply and use of water in African communities. Progress in Water Technology 11, 49-55.

Furuta, K.,Sato, S. & Kawamura, H. (1976). Effect of formaldehyde on disinfection of filtered air under positive pressure (FAPP) type house. Poultry Science 55, 2295-2299.

Gaustad, J.W., McDuff, C.R. & Hatcher, H.J. (1974). Test method for the evaluation of virucidal efficacy of 3 common liquid surface disinfectants on a simulated environmental surface. Applied Microbiology 28, 748-752.

Ginoza, W. (1968). Inactivation of viruses by ionizing radiation and by heat. In Methods in Virology, Vol. 4, K. Maramorosch and H. Koprowski (Eds.). Academic Press, New York, pp. 139-209.

Goh Rowland, S. G. J., Lloyd-Evans, N., Williams, K. & Rowland, M. G. M. (1985). The etiology of diarrhoea studied in the community in young urban Gambian children. Journal of Diarrhoeal Diseases Research 3, 7-13.

Grimwood, K., Abbott, G. D., Fergusson, D. M., Jennings L. C. & Allan J. M. (1983). Spread of rotavirus within families: a community based study. British Medical Journal 287, 575-577.

Grossegebauer, K. (1970). Virus Disinfection. In Disinfection. Benarde, M. A. (Ed.). Marcel Dekker Inc. New York, 1970. pp. 103-148.

Gwaltney, J.M. & Hendley J.O. (1982). Transmission of experimental Rhinovirus infection by contaminated surfaces. American Journal of Epidemiology 116, 828-833.

Gwaltney, J.M., Moskalski, P.B. & Hendley, J.O. (1978). Hand-to-hand transmission of rhinovirus colds. Annals of Internal medicine 88, 463-467.

Hall, C.B. (1983). The nosocomial spread of respiratory viral infections. Annual Reviews of Medicine 34, 311-319.

Hall, C.B., Douglas, R.G.Jr. & Geiman J.M. (1980). Possible Transmission by fomites of Respiratory Syncytial Virus. The Journal of Infectious Diseases 141, 98-102.

Hall, C.B., Douglas, R.G., Geiman, J.M. & Messner, M.K. (1975). Nosocomial Respiratory syncytial virus infections. New England Journal of Medicine 293, 1343-1346.

Halvorsrud, J. & Orstavik, I. (1980). An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scandinavian Journal of Infectious Diseases 12, 161-164.

Hara M., Mukoyama, J., Tsuruhara, T., Saito, Y. & Tagaya, I. (1976). Duovirus in school children with gastroenteritis. Lancet 1, 311.

Harakeh, M.S. (1984). Inactivation of enteroviruses, rotaviruses and bacteriophages by peracetic acid in a municipal sewage effluent. FEMS Microbiology Letters 23, 27-30.

Harakeh, M.S. & Butler, M. (1984). Inactivation of human rotavirus, SA-11 and other enteric viruses in effluent by disinfectants. Journal of Hygiene (Cambridge) 93, 157-163.

Harris, J. R., Cohen, M. L. & Lippy, E. C. (1983). Water-related disease outbreaks in the United States - 1981. Journal of Infectious Diseases

148, 759-762.

Haug, K. W., Orstavik, I. & Kvelstad, G. (1978). Rotavirus infections in families - A clinical and virological study. Scandinavian Journal of Infectious Diseases 10, 265-269.

Hayden, G.F., Deforest, D., Hendley, J.O. & Gwaltney, J.M. Jr. (1984). Inactivation of rhinovirus on human fingers by virucidal activity of glutaric acid. Antimicrobial Agents and Chemotherapy 26 928-929.

Hayden, G.F., Gwaltney, J.M., Thacker, D.F. & Hendley, J.O. (1985). Rhinovirus inactivation by nasal tissues treated with virucide. Antiviral Research 5, 103-109.

Hendley, O.J., Mika, L.A. & Gwaltney, J.M. (1978). Evaluation of virucidal compounds for inactivation of rhinovirus on hands. Antimicrobial Agents and Chemotherapy 14, 690-694.

Hendley, O.J., Wenzel, R.P. & Gwaltney (1973). Transmission of Rhinovirus Colds by Self innoculation. New England Journal of Medicine 288, 1361-1364.

Heiber, J. P., Shelton, S., Nelson, J. D., Leon, J. & Mohs, E. (1978). Comparison of human rotavirus disease in tropical and temperate settings. American Journal of Diseases of Children 132, 853-858.

Hildreth, C., Thomas, M., & Ridgeway, G. L. (1981). Rotavirus infection in an obstetric unit. British Medical Journal 282, 231.

Hoh, H., Presser, W., und Wigand, R. (1983). Nosokomial-Infektion durch Rotaviren bei Erwachsennen. Deutsche Medizinische Wochenschrift 108, 1586-1591.

Holdaway, M. D., Kalmakoff, J., Schroeder, B. A., Wright, G. C. & Todd, B. A. (1982b). Rotavirus infection in Otago: a serological study. New Zealand Medical Journal 95, 110-112.

Holdaway, M. D., Todd, B. A., Schroeder, B. A. & Kalmakoff, J. (1982a). Rotavirus infection in New Zealand. New Zealand Medical Journal 95, 67-69.

Holmes, I.H. (1979). Viral gastroenteritis. Progress in Medical Virology 25, 1-36.

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

Hung, T., Chen, C., Wang, C., Yao, H., Fang, Z., Chou, T., Chao, Z., Ye, W., Chang, X., Den, S., Liang, X. & Chang, W. (1984). Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. Lancet 1, 1139-1142.

Ijaz, M. K. (1985). Studies on the airborne survival of rotaviruses and a human coronavirus. PhD., University of Ottawa, Ottawa, Ontario, Canada.

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

Ijaz, M. K., Sattar, S. A., Johnson-Lussenburg, C. M., Springthorpe, V. S. & Nair, R.C. (1985b). Effect of relative humidity, atmospheric temperature and suspending medium on the airborne survival of human rotavirus. Canadian Journal of Microbiology 31, 681-685.

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

Keenlyside, R.A., Hierholzer, J.C., D'Angelo, L.J. (1983). Keratoconjunctivitis associated with adenovirus type 37: An extended outbreak in an opthlamologist's office. The Journal of Infectious Diseases 147, 191-198.

Keswick, B. H., Pickering, L. K., DuPont, H. L. & Woodward, W. E. (1983a). Survival and detection of rotaviruses on environmental surfaces in day-care centres. Applied and Environmental Microbiology 46, 813-816.

Keswick, B. H., Pickering, L. K., DuPont, H. L. & Woodward, W. E. (1983b). Prevalence of rotavirus in children in day-care centres. Journal of Pediatrics 103, 85-86.

Kim, H. W., Brant C. D., Kapikian, A. Z., Wyatt, R. G., Arrobio, J. O., Rodriguez, W. J., Chanock, R. M. & Parrott, R. H. (1977). Human rotavirus-like agent infection. Occurence in adult contacts of pediatric patients with gastroenteritis. Journal of the American Medical Association 28 404-407.

Kirchoff, H. (1969). Problems of virus disinfection shown with the example of Newcastle disease virus. Deutsche Tierarztliche Wochenschrift 76, 71-74.

Klein, M. & Deforest, A. (1983). Principles of Viral Inactivation. In Disinfection, Sterilization and Preservation. Block, S.S. (Ed.) Lea & Febiger, Philadelphia, PA, U.S.A.

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

Konno, T., Suzuki, H., Kutsuzawa, T., Imai, A., Katsushima, N., Sakamoto, M., Kitoaka, S., Tsuboi, R. & Adachi, M. (1978). Human rotavirus infection in infants and young children with intussusception. Journal of Medical Virology 2, 265-259.

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

Kurstak, E. & Marusyk, R.G. (1984). Introduction: In Control of Virus Diseases. Eds. (Kurstak, E. & Marusyk, R.C.) Marcel Dekker Incorporated. New York, NY.

Kurtz, J.B., Lee, T.W. & Parsons, A.J. (1980). The action of alcohols on rotavirus, astrovirus and enterovirus. Journal of Hospital Infection 1, 321-325.

Larson, T. & Bryson, Y.J. (1985). Fomites and herpes simplex virus. The journal of Infectious diseases 151, 746-747.

Lemp, G.F., Woodward, W.E., Pickering, L.K., Sullivan, P.S., DuPont, H. L. (1984). The relationship of staff to the incidence of diarrhoea in day care centres. American Journal of Epidemiology 120, 750-758.

Lycke, E., Blomberg, J., Berg, G., Eriksson, A. & Madsen, L. (1978). Epidemic acute diarrhoea in adults associated with infantile gastroenteritis virus. Lancet ii, 1056-1057.

Mahl, M.C. & Sadler, C. (1975). Virus Survival on inanimate surfaces. Canadian Journal of Microbiology 21, 819-823.

MacCullum, F.O. & McDonald, J.R. (1957). Survival of variola virus in raw cotton. Bulletin of the World Health Organisation 16, 247-254.

Maiya, P. P., Pereira, S. M., Mathan M. M., Bhat, P., Albert, M. J. & S.J. Baker (1977). Aetiology of acute gastroenteritis in infancy and childhood in southern india. Archives of Diseases in Children 52, 482-485.

Marrie, T. J., Lee, S. H. S., Faulkner R. S., Ethier, J. & Young, C. H. (1982). Rotavirus infection in a geriatric population. Archives of Internal Medicine 142, 313-316.

Mata, L., Simhon, A., Urrutia, J.J., Kronmal, R. A., Fernandez, R. & Garcia, B. (1983). Epidemiology of rotavirus in a cohort of 45 Guatemalan Mayan Indian children observed from birth to the age of three years. The Journal of Infectious Diseases 148, 452-461.

Mathan, M. M., (1984). Role of rotavirus infection in hospitalised children in India. Paper presented at the XI International Congress for Tropical Medicine and Malaria, September 1984, Calgary, Alberta.

McCaustland, K.A., Bond, W.W., Bradley, D.W., Ebert, J.W. & Maynard, J.E. (1982). Survival of hepatitis A virus in feces after drying and storage for 1 month. Journal of Clinical Microbiology 16, 957-958.

McGeady, M.L., Siak, J.S. & Crowell, R.L. (1979). Survival of coxsackievirus B3 under diverse environmental conditions. Applied and Environmental Microbiology 37, 972-977.

McNulty, M.S. & Logan, E.F. (1983). Longitudinal Survey of rotavirus infection in calves. Veterinary Record 113, 333-335.

Medical Research Council (Canada). (1980). Guidelines for the handling of Recombinant DNA molecules and animal viruses and cells.

Meissner, H.C., Murray, S.A., Kiernan, M.A., Snydman, D.R. & McIntosh, K. (1984). A simultaneous utbreak of respiratory syncytial virus and parainfluenza virus type 3 in a newborn nursery. The Journal of Pediatrics 104, 680-684.

Middleton, P. J., Szymanski & Petric, M. (1977). Viruses associated with acute gastroenteritis in young children. American Journal of Diseases in Childhood 131, 733-737.

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

Morens, D. M., Zweighaft, R. M., Vernon, T. M., Garry, G. W., Eslien, J. J., Wood, B. T., Holman, R. C. & Dolin, R. (1979). A waterborne outbreak of gastroenteritis with secondary person-to-person spread. Lancet i, 964-966.

Murphy, A. M., Albrey M. B., & Crewe E. B. (1977). Rotavirus infections of neonates. Lancet ii, 1149-1150.

Nakao, J., Hers, R.G., Bachman, P.A. & Mahnel, H. (1978). Inactivation of transmissible gastroenteritis (TGE) virus of pigs. Berliner und Munchener Tierarztliche Wochenschrift 91, 353-357.

Narang, H.K. & Codd, A.A. (1981). Frequency of preclumped virus in routine feacal specimens from patients with acute nonbacterial gastroenteritis. Journal of Clinical Microbiology 13, 982-988.

Narang, H.K. & Codd, A.A. (1983). Action of commonly used disinfectants enteroviruses. Journal of Hospital Infection 4, 209-212.

Nerurkar, L.S., West, F., May , M., Madden, D.L. & Sever, J.L. (1983). Survival of herpes simples virus in water specimens collected from hot tubs in spa facilities and on plastic surfaces. Journal of the American Medical Association 250, 3081-3083.

Noone, C. & Banatvala, J.E. (1983). Hospital acquired rotaviral gastroenteritis in a general paediatric unit. Journal of Hospital Infection 4, 297-299.

Parkinson, A.J., Muchmore, H.G., Scott, E.N. & Scott L.V. (1983). Survival of human parainfluenza viruses in the south polar environment. Applied and Environmental Microbiology 46, 901-905.

Paul, M. O. & Erinle, E. A. (1982). Influence of humidity on rotavirus prevalence among Nigerian infants and young children with gastroenteritis. Journal of Clinical Microbiology 15, 212-215.

Pattison, C.P., Boyer, K.M., Maynard, J.E. & Kelly, P.C. (1974). Epidemic hepatitis in a clinical laboratory. Possible association with computer card handling. Journalof the American Medical Association 230, 854-857.

Perez-Schael, I., Daoud, G., White, L., Urbina,G., Daoud, N., Perez, M. & Flores, J. (1984). Rotavirus shedding by newborn children. Journal of Medical Virology 14, 127-136.

Petersen, N. J., Collins, D. E. & Marshall, J. H. (1973). A microbiological assay technique for hands. Health Laboratory Science 10, 18-22.

Phillips, C.A. & Grim, C.A. (1965). Comparison of the elution and adsorption of picornaviruses by cotton and calcium alginate wool swabs. Applied Microbiology 13, 457-459.

Pickering, L. K., Evans, D. G., Dupont, H. L. & Vollet III, J. J. (1981). Diarrhoea caused by Shigella, rotavirus and giardia in day-care centres: Prospective study. Journal of Pediatrics 99, 51-56.

Ramia, S. & Sattar, S. A. (1979). Simian rotavirus SA-11 plaque formation in the prescence of trypsin. Journal of Clinical Microbiology 10, 609-614.

Ramia, S. & Sattar, S. A. (1980). Concentration of simian rotavirus SA-11 from potable waters using talc-Celite layers and hydroextraction. Applied and Environmental Microbiology 39, 493-499.

Raphael, R.A., Sattar S.A. & Springthorpe V.S. (1983). Rotavirus inactivation by chemical disinfectants and antiseptics used in microbiology laboratories. Proceedings Association for Biological Safety Conference, May-June, 1983, Ottawa, Ontario. pp.170-183.

Raphael, R.A, Sattar, S.A. & Springthorpe V.S. (1985). Long-term survival of human rotavirus in raw and treated river water. Canadian Journal of Microbiology 31, 124-128.

Reagan, K.J., McGeady M.L. & Crowell, R.L. (1981). Persistence of Human Rhinovirus Infectivity Under Diverse Environmental Conditions. Applied and Environmental Microbiology 41, 618-620.

Reed, S. (1975). An investigation of the possible transmission of rhinovirus colds through indirect contact. Journal of Hygiene Cambridge 75, 249-258.

Ringertz, O. & Zetterberg, B. (1967). Serum hepatitis among Swedish trackfinders. An epidemiological study. The New England Journal of Medicine 276, 540-546.

Rocchi, G., Vella, S., Resta, S., Cochi, S., Donelli, G., Tangucci, F., Menichella, D., Varveri, A. & Inglese, R. (1981). Outbreak of rotavirus gastroenteritis among premature infants. British Medical Journal 283, 886.

Rossi, A., Agliano, A.M., Spanu, T., Salvaggio, E., Rossodivita, A., Chezzi, C. & La Monica, S. (1982). Incidence of rotaviruses and astroviruses in children without symptoms of gastroenteritis. Igiene Moderna 78, 230-239.

Rowland M.G.M, Goh, S.G.J., Williams, K., Campbell, A.D., Beards, G.M., Sanders, R.C. & Flewett, T.H. (1984). Annals of Tropical Paediatrics 5, 23-28.

Russell, A.D., Ahonkhai, I. & Rodgers, D. T. (1979). microbiological applications of the inactivation of antibiotics and other antimicrobial agents. Journal of Applied Bacteriology 46, 207-245.

Ryder, R. W., McGowan, J. E., Hatch, M. H. & Palmer E. L. (1977). Reovirus-like agent as a cause of nosocomial diarrhoea in infants. Journal of Pediatrics 90, 698-702.

Samadi, A. R., Huq, M. I. & Ahmed, Q. S. (1983). Detection of rotavirus in the handwashings of attendants of children with diarrhoea. British Medical Journal 286, 188.

Salmi, T. T., Arstila, P. & Koivikko, A. (1978). Central nervous system involvement in patients with rotavirus gastroenteritis. Scandinavian Journal of Infectious Diseases 10, 29-31.

Santosham, M., Yolken, R.H., Quiroz, E., Dillman, L., Oro, G., Reeves, W. & Sack, B. (1983). Detection ofrotavirus in respiratory secretions of children with pneumonia. Clinical and Laboratory Observations 103, 583-585.

Sattar, S. A., Ijaz, M. K., Johnson-Lussenburg M. & Springthorpe, V.S. (1984). Effect of relative humidity on the airborne survival of rotavirus SA-11. Applied and Environmental Microbiology 47, 879-881.

Sattar, S. A., Raphael, R. A., Lochnan, H. & Springthorpe, V.S. (1983). Rotavirus inactivation by chemical disinfectants and antiseptics used in hospitals. Canadian Journal of Microbiology 29, 1464-1469.

Schumann, K-O. & Grossgebauer, K. (1977). Experiments on disinfection of

vaccinia virus embedded in scabs/or at the hand. Zentralblatt fur Bakteriologie und Hygiene I. Abt. (Orig.B) 164, 45-63.

Schurmann, W. & Eggers, H.J. (1983). Antiviral activity of an alccholic hand disinfectant: Comparison of the in vitro suspension test with in vivo experiments on hands, and on individual fingertips. Antiviral Research 3, 25-41.

Selwyn, S. (1965). The transmission of bacteria and viruses on gummed paper. Journal of Hygiene, Cambridge 63, 411-416.

Sidwell, R.W., Dixon, G.J. & McNeil, E. (1966). Quantitative studies on fabrics as disseminators of viruses. I. Persistence of vaccinnia virus on cotton and wool fabrics. Applied Microbiology 14, 55-59.

Sidwell, R.W., Dixon, G.J. & McNeil E. (1967). Quantitative studies on fabrics as disseminators of viruses. III Persistence of vaccinia virus on fabrics impregnated with a virucidal agent. Applied Microbiology 15, 921-927.

Sidwell, R.W., Westbrook L., Dixon G.J. & Happich W.F. (1970). Potentially infectious agents associated with shearling bedpads. I Effect of laundering with detergent-disinfectant combinations on polio and vaccinia viruses. Applied Microbiology 19, 53-59.

Sitbon, M., Lecerf, A., Garin, Y. & Ivanoff, B. (1985). Rotavirus prevalence and relationships with climatological factors in Gabon, Africa. Journal of Medical Virology 16, 177-182.

Slobodenyuk, V.K. & Karpukhin, G.I. (1970). Experimental basis for the aerosol method of disinfection in virus infections. 11. The inactivating effect of aerosols of hydrogen-peroxide, chloramine and hexylresorcinol on different viruses in the air and on surfaces. Zh. Mikrobiol. Epidemiol. Immunobiol 47, 113-117.

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

Sporkenbach, J., Wiegers, K.J. & Dernick, R. (1981). The virus inactivating efficacy of peracids and peracidous disinfectants. Zentralblatt fur Bakteriologie und Hygiene (Orig. B) 173, 425-439.

Springthorpe, V.S., Grenier, J.L., Lloyd-Evans, N. & Sattar, S.A. (1986). Chemical disinfection of human rotaviruses: Efficacy of commercially-available products in suspension tests. Journal of Hygiene. In press.

Steinhoff, M.C. & Gerber M.A. (1976). Rotavirus infection in neonates. Lancet 1, 775.

Stellmacher, W., Schwebs, M. & Somnitz M. (1973). Effect of temperature on solutions of disinfectants. Arch. Exp. Veterinaermed 27, 341-347.

Stintzing, G., Back, E., Tufvesson, B., Johnsson, T., Wadstrom, T & Habte, D. (1981). Seasonal fluctuations in the occurence of enterotoxigenic bacteria and rotavirus in paediatric diarrhoea in Addis Ababa. Bulletin of the World Health Organisation 59, 67-73.

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

Tan, J. A. & Schnagl, R. D. (1981). Inactivation of a rotavirus by disinfectants. Medical Journal of Australia 1, 19-31.

Tan, J. A. & Schnagl, R.D. (1983). Rotavirus inactivated by a hypochlorite-based disinfectant: a reappraisal. The medical Journal of Australia 3, 550.

Totterdell, B. M., Chrystie, I. L. & Banatvala, J. E. (1976). Rotavirus infections in a maternity unit. Archives of Diseases in Childhood 51, 924-928.

Thraenhart, O. & Kuwert, E. (1977). Virucidal efficacy of a chemical instrument surface disinfectant ("Gigasept") against coxsackie virus type B3. II Investigation in the instrumental surface test. Zentralblatt fur Bakteriologie Miicrobiologie und Hygiene. 1.Abt. Orig. B. 164, 22-44.

Trouwborst, T., Kuyper, S., de Jong, J.C. & Plantinga, A.D. (1974). Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. Journal of General Virology 24, 155-165.

Turner, R., Shehab, Z., Osborne, K. & Hendley, J.O. (1982). Shedding and survival of herpes simplex virus from 'fever blisters'. Pediatrics 70, 547-549.

Turner, F.J. (1983). Hydrogen peroxide and other oxidant disinfectants. In Disinfection, Sterilization and Preservation. Block, S.S. (Ed.), Lea & Febiger, Philadelphia, 1983. pp. 240-250.

Wallbank, A. M., Drulak, M., Poffenroth, L., Barnes, C., Kay, C. & Lebtag, I. (1978). Wescodyne: lack of activity against poliovirus in the presence of organic matter. Health Laboratory Science 15, 133-137.

Wallis, C., Behbehani, A. M., Lee, L. H. & Bianchi, M. (1963). The ineffectiveness of organic iodine as a viral disinfectant. American Journal of Hygiene 78, 325-329.

Ward, R.L. & Ashley C.S. (1977). Effects of waste-water sludge and its detergents on the stability of rotavirus. Applied and Environmental Microbiology 39, 564-579.

Wenman, W.W., Hinde, D., Feltham, S. & Gurwith, M.D. (1979). Rotavirus infection in adults. Results of a prospective family study. The New England Journal of Medicine 301, 303-306.

West, D.J. (1984). The risk of hepatitis B infection among health professionals in the United States: A Review. American Journal of Medical Science 287, 26-33.

Williams, F.P. (1985). Membrane associated viral complexes observed in stools and cell culture. Applied and Environmental Microbiology. 50 522-526.

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

Yolken, R. H., Bishop, C. A., Townsend, T. R., Bolyard, E. A., Bartlett, J., Santos, G. W. & Saral, R. (1982). Infectious gastroenteritis in bone -marrow transplant recipients. New England Journal of Medicine 299, 1156-1161.

Yolken, R. & Murphy, M. (1982). Sudden infant death syndrome associated with rotavirus infection. Journal of Medical virology 10, 291-296.