A study on the use of disinfectants against pseudorabies

virus under selected environmental conditions

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ABSTRACT

Disinfectants are used widely in farm practice as control
and preventive measures against disease agents. This study
was conducted to assess the activities of four categories of
disinfectants in the presence of some natural environmental
factors that were present in animal raising farms against
pseudorabies virus (PRV) - a herpes virus. The four cate-
gories of disinfectants included chlorhexidine (Nolvasan[R]),
quaternary ammonium compound (Roccal[R]), phenol derivatives
(1 Stroke Environ[R]), and alkali (lye). The environmental
fomites that were tested were concrete, wood, plastic grate,
soiled bedding and loam soil.

The eighth passage of S62/26 strain of PRV was pre-
pared and stored at -90°C in 1 ml vials. The virus sus-
pensions were thawed and diluted 1:10 in physiological saline
solution (PSS) as suspending fluid. Tests were then per-
formed using PSS, saline G, porcine saliva and porcine nasal
washings as diluents with which the virus interacted in the
presence of environmental fomites and disinfectants.

The disinfectants were initially diluted to 10X the
manufacturers' recommended concentrations for field use
(RCFU) with distilled water, and from these dilutions, initial
decimal dilutions at 10^{-1}, 10^{-2} and 10^{-3} were made in PSS to
give final test dilutions of 1X, 0.1, 0.01 and 0.001 from the

RCFU.

The environmental factors interacting with the virus in this study; i.e., fomites, disinfectants and diluents, were used in a ratio of 1:1:1:7 respectively, by adding 0.5g fomites, 0.5 ml of appropriate disinfectant solutions, and 0.5 ml virus solution to 3.5 ml of the test diluents for 5 or 30 min. reaction time at two temperatures, 4° and 25°C.

The activities of the disinfectants against PRV were studied in the presence of 7 environmental fomites in 4 diluents and at 2 environmental temperatures. Test conditions were rigorously controlled and all assays were performed by plaque reduction techniques in MDBK cells cultures.

Clean, weathered concrete exerted an alkaline pH effect (11.8 $\pm$ 0.4) in the presence of the 4 diluents tested, inhibiting the virus by itself and greatly enhancing the 4 disinfectants activity at both temperatures tested. Plastic grate showed a mild inhibitory effect on the virus, especially in saliva and this inhibitory effect improved the effectivity of the 4 test disinfectants as well. New wood had an inactivating effect on PRV by its acid pH (5.2 $\pm$ 0.4) in the presence of PSS, saline G and nasal washings. However, when disinfectants were applied, new wood protected the virus from their action, inhibiting by >90% the 4 disinfectants in these diluents. In the presence of saliva,

however, new wood lost its inactivating activity for the virus while still protecting it from the 4 disinfectants activities. Soiled bedding inhibited by >90% the activities of Nolvasan and Roccal, and unless maintained at RCFU levels, these two disinfectants were not found effective for soiled bedding disinfection. I Stroke Environ and sodium hydroxide were still effective if applied in concentrations not $<\frac{1}{10}$ of their RCFU levels. Loam soil tied up Nolvasan and Roccal activities in the presence of the 4 diluents, and only I Stroke Environ and sodium hydroxide were effective if they were used in concentrations not $<\frac{1}{10}$ of their RCFU.

Thus, concrete and plastic grate improved disinfectant activities while new wood, plastic grate and loam soil inhibited the 4 disinfectants. In the absence of interfering fomites, Nolvasan was more effective at 25°C than at 4°C, but no significant differences were noted in the activities of the other 3 disinfectants at either temperature. I Stroke Environ was more effective when used at 25°C than at 4°C to disinfect new wood. All 4 disinfectants were more effective at 4°C than at 25°C for disinfecting loam soil.

INTRODUCTION

The chain of transmission of any infectious disease, in-
cluding pseudorabies, includes the presence of an infected
host, escape of the infective agent from the host, the
survival of the agent in the environment outside the host,
the transport of the agent to a susceptible host, the
entrance of the agent into this susceptible host, and the
establishment of infection in this host. Efforts to pre-
vent and to control infectious diseases are directed toward
the links in this chain of transmission.

At critical points, attempts are made to break the chain
by treating the infected host, by preventing escape of the
infective agent from the host, by attacking its survival
outside the host, by preventing its transport to and entry
into a susceptible host and by preventing establishment of
infection in the new host. Chemical, biological and environ-
mental manipulations are most used in these attempts.

The goal of this study was to assist in interrupting
the survival of pseudorabies virus in the environment.
Experimental tests were directed toward the use of chemical
disinfectants to prevent survival of pseudorabies virus
outside the living host when suspended in body fluids and
in contact with environmental fomites typical of the farm
settings in which pseudorabies virus may be quite affected

by the organic or inorganic materials in the environment
into which its falls. The infective virus also escapes from
the host with different types and quantities of organic
materials, depending on its route of excretion. In this
study, assessment was made as to how much and how long such
organic materials and surrounding fomites could support
the infective agent in the environment and how effective
commonly used types of disinfectants could be in inactivating
the virus in these environmental conditions to prevent its
entry and infection into new susceptible hosts.

Pseudorabies virus, an enveloped herpes virus, causes
heavy economic losses to the livestock industries of many
countries. Under environmental conditions present in these
countries, the virus has been found to survive in the en-
vironment for extended periods of time, often in spite of
attempts to destroy it. Although the virus has been
described as sensitive to many kinds of chemical disinfec-
tants, experiences on infected farms have not been so uni-
formly successful in their use. The 4 disinfectants tested
under a variety of environmental conditions in this study were
representative of chlorhexidine, quaternary ammonium, phenolic
and alkaline disinfectant compounds.

GENERAL FAMILY CHARACTERISTICS

OF THE HERPETOVIRIDAE

The Herpetoviridae is a family of viruses which includes pathogens for human beings as well as for a variety of wild and domestic animals. The virions have linear double stranded deoxyribonucleic acid (DNA) cores of 70 to 150 x 10^6 daltons molecular weight with a diameter of 75-77.5 nm enclosed in icosahedral capsids of 162 capsomers with a diameter of 90-105 nm. The nucleocapsids are coated with outer envelopes which render the precise definition of their diameter difficult, ranging between 150-200 nm. Within a population of virions, many particles do not possess envelopes and some are empty capsids (29, 47, 68).

The purified enveloped viral particles of herpes viruses, such as Herpes simplex, contain 25-30 virus specified proteins which constitute up to 70% of the virions (47). The DNA molecules which comprise approximately 7%, and 20-22% are host specified envelope phospholipids (47). Polyamines in small amounts are also found as spermine in the nucleocapsids and spermidin within the envelopes, as well as virus specific subunit glycoproteins which are also contained in the envelopes (42, 47).

Due to the presence of lipids in their envelopes, herpes

viruses are sensitive to chemical and physical agents (42, 68, 90), being readily inactivated by lipid solvents, variations of pH and by heat; also they are unstable at room temperature (42, 47, 90).

The Hereptoviridae family is divided into three subfamilies; Alphaherpesvirinae (Herpes simplex), Beta-herpesvirinae (Cytomegalovirus) and Gammaherpesvirinae (Epstein-Barrvirus) antigens among members within the same genera which may be detected by several nonneu-tralizing immunological tests. Pseudorabies virus has been shown by gel immunodiffusion and by immunofluorescent techniques to have antigenic relations with Herpes simplex I and II, Herpes simiae (B-virus) and to lesser extents, with Marek's disease virus, turkey herpesvirus (44, 45, 66), and infectious bovine rhinotracheitis (IBR) virus (Reed, D. and K. Platt, personal communication, Department of Veterinary Microbiology and Preventive Medicine, ISU, Ames, Iowa, 1981).

The herpes viruses are characterized biologically by the formation of latent infections, which may be related to this intranuclear replication, though the mechanism of latency has not been well determined (29, 47). In laboratory cell cultures, they are characterized by cyto-pathic destruction of the infected cells and the formation of plaques or pocks which differ among virus strains (29, 47).

Characteristics of Pseudorabies Virus

Pseudorabies virus (PRV) was first reported by
Schmiedhoffer in Germany in 1910 as the causative agent of
the disease described in swine by Aujeszky in Hungary in
1902 and identified as Aujeszky's disease (AD) or as it had
been called earlier in Europe, pseudorabies (PR) (68, 74).

PRV isolated from swine was found to be neutralized
by serum from mad-itch convalescent cattle, and reciprocally
mad-itch virus was shown to be neutralized by PR convalescent
serum (105). These findings led to the conclusion that the
causative agents of both diseases was one virus with two
immunologically identical strains (106). In 1962, Andrews
used the binomical name Herpesvirus suis for this virus, the
only known herpesvirus to naturally affect swine (68).

A wide range of mammalian cell cultures have been used
to cultivate or isolate PRV. PRV produces type A cowdry
intranuclear inclusion bodies with plaque formation due to
cytopathic effect (cpe) in cell cultures (47, 68). Charac-
teristics of the cpe depends largely on the strain of the virus
virus (96, 97). The correlation between the pathogenicity of
field strains of PRV and their ability to cause cell fusion-
syncytia formation was studied previously (18), and it was con-
cluded that strains of PRV possessing high pathogenicity for
swine and cattle will regularly be syncytia-forming. In

another study (99), five isolates from cattle were examined

with respect to number of properties, including cpe and found

to be nonsyncytia-forming, and concluded that they were atten-

uated. Golais and Sabo (50) studied as many as 10 virulent and

4 attenuated strains. Dow, in Ireland, also reported (37)

that four strains existed: NIA1 (5 to 20 percent mortality),

NIA2 (10 to 20 percent mortality), NIA3 (10 to 100 percent

mortality) and NIA4 (no mortality, useful for making vaccine).

Mayer and Skoda tested (87) two modified and four virulent

strains of PRV for behavior at different temperatures.

The optimum pH and temperature stability were reported

to be between pH 6 and 8; the greatest temperature

stability of unfrozen virus was at 4°C and of frozen virus

between -80°C and -90°C; at -13°C the virus was inactivated

rapidly (34).

Pseudorabies Disease

Also called Aujeszky's disease, mad-itch, and infectious

bulbar paralysis, PR is an acute infectious disease of swine

and many other mammals. Under farm conditions, the virus has

been found to cause disease in swine, cattle, sheep, dogs and

cats (40, 62, 91). Although goats were excepted by some (65),

they have been included in the lists of others (46). There

is some doubt about natural susceptibility of the horse.

Deer, foxes, mink, badgers, coyotes, skunks, racoons, opossum, rats, mice and rabbits have been listed as susceptible to natural infection (12, 54).

Swine are highly susceptible to PRV, developing peracute clinical disease with nearly 100% mortality during the first few weeks of life, but as they become older, they develop greater resistance to the clinical disease, especially to that caused by some strains (53, 58). It has been reported (120) that in some herds of swine the acute form of PR has never appeared and no visible signs of PR have been seen; yet, swine on these farms exhibit positive serum neutralization titers indicating the virus to be present on those farms, often in a latent form for a long period (2 to 3 years or more). PR infections in pigs involve the respiratory, nervous and reproductive systems; the principal manifestations are encephalitis and reproductive failures. Local pruritis may be present but is less marked in pigs than in most other species; occasionally infected cattle may not develop pruiritis (15, 24). Domestic and wild animals of several species, other than swine, are highly susceptible to infection and develop severe clinical disease but the disease in them is usually of a noncontagious nature (55, 56). Unless carcasses of these animals are directly or indirectly consumed by pigs, infections in these species are likely to be dead end. All recovered

swine must be considered latently infected (22, 53, 54, 93, 120). While production of latent infection in organ cultures of species other than swine has been developed in the laboratory, the development of latent infection in occasional animals other than swine which recover from PR has not been determined (47).

EPIDEMIOLOGY OF PSEUDORABIES

It has been reported (57) that "Mad itch" in cattle had

been recorded in the United States (U.S.) on September 10,

1813; that is about 90 years before identification of the

disease in Hungary by Aujeszky. Also, it was reported (52)

that despite the sickness and death caused to a wide range

of animal species, not much attention was paid to the disease

in the U.S. until the late months of 1962 when increasing

losses in swine and domestic animals associated with them,

including cattle, sheep, dogs and cats, began to be observed

in the Midwest. The severity and spread of the disease in

swine in the U.S. were similar to what had been observed for

decades in Europe, including England, Ireland, Hungary,

Netherlands, Germany, Denmark and Yugoslavia (90) (Kluge,

John, Department of Veterinary Pathology, Iowa State Uni-

versity, Ames Iowa, personal communication). The disease

has been recognized and reported (18, 46) from most

European countries, Asia, North Africa, South America, as

well as North America and recently New Zealand. In the U.S.,

Stewart (112), reporting on the widespread nature of the

disease, noted that during the period from fiscal year 1966

through fiscal year 1972, the National Veterinary Services

Laboratory, Animal and Plant Health Inspection Services

(NVSL, APHIS), U.S. Department of Agriculture, had diagnosed

PR disease in pigs in eleven states. From the states men-
tioned during the "Pseudorabies Fact Finding Conference"
April 4-5, 1977, in the U.S., major outbreaks were re-
ported in Iowa, Illinois, South Dakota, Oklahoma, Missouri
and Indiana.

Swine play the major role in contracting and spreading
the disease as they have been found to be the only reservoir
host (8, 10, 11, 12, 53, 55, 91, 118).

PR is usually contagious among swine only; other animals
in general, contract the disease when they come into contact
with pigs or their contaminated byproducts. Cattle, sheep,
dogs and cats, as well as other domestic and wild animals
have been found to become infected when they contacted infec-
ted swine (15, 21, 24, 63, 111, 114, 118, 120, 121). Trainer and
Karstad (115) mention the possibility that wild mammals, such
as foxes, skunks, muskrats, rabbits, raccoons, badgers,
woodchucks, oppossum and deer could play a role in mechanical
transmission of the virus through their carcasses, as most
of these animals died before active shedding of virus could
lead to transmission, but the virus was present in so many
tissues of these animals that transmission could occur when
their carcasses were consumed directly or indirectly by
pigs. A study (123) on the possible transmission of PRV by
raccoons concluded that although raccoons were susceptible

only to moderately large doses of virus (10^4 to 10^5 $TCID_{50}$)
their carcasses could be a source of transmission. Recent
studies at Purdue University have shown that PRV can be
transmitted from pigs to raccoons and from raccoons to pigs
through contact exposure, by eating at the feed trough and
eating infected carcasses. Virus shed in saliva from infected
raccoons was shown to contaminate feedstuffs which could then
serve as a source of infection for ruminant animals (67).
The same study also indicated that PRV may be shed by in-
fected cattle and sheep. Animal species, other than swine,
are not considered to be reservoir hosts, however, as there
is no direct lateral transmission of the virus in these
species (7, 8, 16, 17, 53). Generally, pigs play the major
and frequently the only role in the transmission and spread of
the disease, with the role of any other species being
transient and over short distances only (53, 61, 81). In
swine, vertical as well as coital transmission have been
demonstrated (81). Swine display different clinical signs,
depending upon their ages at the infection time, the virus
strain involved and management of the herd (53, 81). The
disease is usually fatal to swine only in their first few
weeks of age (53). Infected adult swine may show mild
clinical symptoms but the infection is usually unnoticed,
and the animals become latent carriers without going through

any clinical phase (42, 46, 49, 81, 108, 120).

Contact transmission usually occurs by inhalation by
susceptible pigs, directly into the moist nasal orifices,
of virus exhaled or in oral secretions of clinically in-
fected or carrier pigs, or indirectly from contaminated food
or fomites on which virus has been deposited from the
nasopharynx of clinically infected or carrier swine. The
incidence of PR in swine has been reported to occur more
frequently in large units than in smaller ones, due to
increased contact between infected and susceptible hosts (1).
The disease frequency has been found to increase in winter
and early spring (69), which also is considered to be due to
housing the animals in greater confinement with reduced
air flow during the winter, resulting in increased respira-
tory contact. In 1972, Baskerville (6) reported that a
minimum infective dose of virus of 10 $TCID_{50}$ could initiate
the disease but fever did not develop until the eighth day
postinoculation. It was also reported (69) that specific-
pathogen-free (SPF) pigs 36-days of age when inoculated with
10^3 plague-forming units (PFU)/ml of PRV, developed clinical
signs within 48 to 72 hours, and six of fifteen died in the
first week (5 to 8 days postinoculation).

During the clinical phase, the virus may be found to
be shed through nasal and oral discharges (52, 76, 88),

especially saliva, from the first day of illness up to 17 days after onset (22, 76, 89). In addition to nasal and oral secretion of PRV, some workers have reported the isolation of the virus from urine, feces, milk and from boars at service (52, 75, 89). Others were not able to isolate PRV from urine or feces of affected animals (106), although the virus may be recovered from mucosa of the ileum and urinary bladder of such animals (13, 33, 85, 88, 107). Nikitin had been quoted (88) as having isolated the virus from urine for up to 186 days following clinical recovery. In infected boars, PRV has been recovered from prostatic and seminal vesicle fluids and from the prepuce (89).

Persistent carrier swine have been found to shed the virus for long periods following the first episode or time of infection (13, 47, 49, 79, 89, 124). In one study, it was reported (13) that the virus had been recovered from infected sows after 6 weeks to 13 months. In another study, PRV was shed by a sow that had been clinically infected 19 months earlier (33). In an additional study (114), the virus was isolated from a carrier sow 6 months after the initial episode. Cartwright in England, reported (Kluge, J, Department of Veterinary Pathology, Iowa State University, Ames, Iowa, personal communication), the isolation of PRV from a subclinically infected herd after more than

two years.

This carrier state or virus latency in infected pigs resembles infection by other herpesviruses in epizootiology, clinical signs, and pathological changes, including Herpesvirus B, Herpes simplex and infectious bovine rhinotrachitis viruses (46, 93, 109). Vaccination of swine with either attenuated live or inactivated vaccines has not eliminated the carrier state or prevented shedding of the virus by carrier pigs. However, vaccination has decreased the clinical signs and greatly reduced mortality in epidemic infection (1, 9, 21, 26; 84; 93, 114, 124).

ECONOMIC IMPACT OF PSEUDORABIES

Beran et al. (14) studied the economic impact of one epidemic in Iowa which extended for 16 months in three interrelated foci, to be about $0.5 million or around $28,912 per farm outbreak. Bedoya (10) reported the disease problem in Mexico when the first serious outbreak was recorded in swine in 1973; he noted that the disease killed 3500 piglets and in 1975 that it infected 3700 sows and killed 20,000 piglets. In the U.S., the disease is increasing in its cost impact; in 1974 there were 125 reported farm outbreaks, in 1975, 225 farm outbreaks, in 1976, 714 farm outbreaks, and in 1977, 1245 farm outbreaks. There were 535 farms quarantined in Iowa alone during the first 10 months in 1978. The estimated cost to the industry in 1977 was $21 to $25 million annually (March 22, 1978 APHIS news release). The USDA-APHIS (June 29, 1978) summarized the current threat of this disease to the U.S. swine industry, stating the following; "The number of outbreaks of PR is not only increasing at an alarming rate but financial losses to owners of infected herds are skyrocketing. Dollar losses in individual commercial swine herds have reached as high as $90,000 a year. One university researcher estimated producer losses to be between $21 million and $25 million per year. No estimate has been

made of damage done to the industry by losses in herds of
seed stock producers, whose blood lines constantly add to the
productivity of commercial herds. The spread of PR is ex-
pected to continue if unchecked by action programs. It has
been estimated by the Swine Diseases staff that 60-70
percent of the nation's swine herds could be infected by
1982 if no control program is instituted. This figure
was developed taking into consideration the rate of increase
of seropositive swine surveyed in 1974 and 1977, and the in-
fection rates of European nations where PR is endemic.
Furthermore, since the disease will tend to infect a higher
percent of the larger herds than of the smaller herds, 80
percent of our swine will originate in PR infected herds."

It was reported (1) that "after 1970, there was an in-
crease in size of farms and also a marked increase in the
incidence of A.D. The mean size of the farms rose from 20
sows in 1970 to 50 in 1978. In the beginning, it was the
breeding herds that were the more seriously affected. Now,
the major problems are in fattening herds; especially in the
large ones with more than 1000 fatteners." He also added,
that A.D. will reduce the mean daily growth rate by 30 to 50
gm and will increase the average mortality from 2% to 5% in
fattening units.

The estimates of economic costs of PR to the swine

industry do not include the losses in animals other than swine, i.e., cattle, sheep, goats, dogs, and cats which come in contact with swine or of fur-bearing animals and other wild animals which succumb to the disease. As mentioned in the previous sections, swine play the major role in the dissemination of the disease and are the sources for the high losses to cattle and sheep farms (15, 24, 54, 74, 114, 119, 121).

It has been reported in the U.S., that the disease has reached the point where it represents a substantial loss to the swine industry and to the animal industry in general, and evidence indicates that the losses will continue to increase annually and that it will probably become more economically significant before effective methods of control are established (89, 124).

The greatest losses from PR are caused by the death of neonatal pigs (79). Mortality in baby pigs has varied from 1 or 2 pigs in a litter to 90% of the baby pigs in the herd at the time of the outbreak. Mortality in older animals is usually about 1-5%. In addition to losses by mortality and interruption of weight gain, PR has been shown to be a cause of reproductive problems in swine including abortions, mummified fetuses and stillborn pigs (22, 23, 72, 85).

PSEUDORABIES VIRUS SURVIVAL OUTSIDE

THE LIVING HOST

Many research workers have studied, most in a limited manner, the survival of PRV outside the living host and have reported some effects of different environmental factors involved in enhancing the spread of the disease by protecting the virus excreted in the external environment by sick or symptomless carrier animals.

PRV was studied outside the living host at 25°C (44), and its survival was found to be dependent on the diluent and fomite combinations in which the virus was suspended or with which it came into contact. When nasal washings and saliva were used as diluents, PRV was inactivated within <2 days and <4 days, respectively, whereas in saline G and phosphate buffered saline solution (PBS), the virus survived for 58 days with minimal (<1 log) inactivation. When PRV was suspended in saline G and placed in contact with selected environmental fomites, the virus was found to survive for up to 7 days on steel and whole corn; for <7 days on plastic and rubber; for <5 days in meat and bone meal; for <4 days in soil, for <3 days on straw and pelleted food; and for <1 day on each of concrete, wood, alfalfa, greengrass and denim cloth. PRV suspended in nasal washings placed in contact with these fomites was

found to be inactivated in <4 days on straw; in <3 days on plastic; in <2 days on each of steel, rubber, soil, whole corn, meat and bone meal; and in <1 day on concrete, wood, alfalfa, pelleted feed, green grass and denim cloth. PRV suspended in porcine saliva as diluent and placed in contact with these fomites was inactivated in <7 days in soil; in <4 days on each of steel, concrete, whole corn, and straw, in <3 days on plastic, in <2 days on each of wood, meat and bone meal, green grass and rubber; and in <1 day on each of alfalfa, pelleted feed, and denim cloth. The same author found PRV to be inactivated within 2 to 7 days in nonchlorinated water and in <1 day in chlorinated water. PRV suspended in fresh swine feces or in effluent from swine pits or from lagoons was inactivated in <2 days, 1 day, or 2 days, respectively. In sow urine uncontaminated with feces, PRV remained at infective levels for 2-week-old pigs after 14 days. Other environmental factors, such as the effect of pH level and temperature on PRV, have been studied (34), and it has been found that the survival of PRV is strongly pH and temperature dependent. At 37°C, at an optimum pH level 6 to 8, the rate of inactivation was 0.6 log per day while at 4°C it was 0.04 per day. These authors found no apparent effect from fluctuation of the temperature between 6°C and 37°C upon the titer of the virus compared to a steady 37°C temperature. At a -13°C steady temperature,

the virus was rapidly inactivated at all pH levels. For

long term storage, it was recommended that the pH should be

adjusted between 6 and 7 and the temperature kept at -90°C

(34). Drying on glass and on gelatin, as well as exposure to

ultraviolet light, quickly inactivated the virus at all pH

levels (34). PRV was also found to be stable at pH levels be-

tween 5 and 9 at 4°C and -60°C, and unstable at -15°C as well

as when subjected to alternate freezing and thawing (125).

Other workers recorded 5 days survival of PRV in slurry in

summer when the temperature was 12 to 18°C, pH level was 6.8

to 7.8 and humidity was 95 to 87% (98). PRV in fermented

edible waste material has been reported to be inactivated

at 20°C and 30°C within 24 hours, but the virus has sur-

vived for 48 hours at 10°C and for 96 hours at 5°C (122).

On contaminated straw and feeding troughs, PRV has been

reported to have survived more than 10 days at 24°C, for as

long as 30 days at 18°C, and in carcasses or on contaminated

wooden boards for 5 or 7 weeks, respectively (84). PRV

was found to be inactivated in less than a month at -18°C

but at 4°C survived for more than 6 months (84). The virus

has been reported to have survived on hay, wood and food for

up to 46 days (81). Others have reported (28, 46) that

the virus was found to survive for 30 days in summer and

46 days in winter.

Viability of PRV was also studied (110) in fodder,

meadow hay, straw, oats, bran, potatoes, sacking, cord,
boards and iron. In contact with these fomites, PRV becomes
inactive in early spring between 32-46 days; in summer in
30 days (mean temperature 24.6°C); in the fall in 15-30 days
(temperature 14.5°-18.5°) and in winter, between 40-46 days
(mean temperature -8.4°C). These authors (110) also reported
that environmental survival of the virus depended on cli-
matic factors in various periods of the year, and that during
sharp fluctuations in temperature, the virus become inactive
earlier than during the absence of such fluctuations.
Survival for 2-7 weeks in an infected environment and for up
to 5 weeks in meat has been reported (22). PRV capability
of surviving in infected premises and in dried tissues were
reported (65) to be 2 months and several months, respective-
ly. In the urine of pigs or sheep, PRV was found (3) to
lose 3 logs of titer in 53 days at 4°C, and to lose the same
in 18 days at room temperature.

Survival of PRV on wood alone and on wood soiled with
manure was studied (117) under different seasonal tempera-
tures. During the summer when the mean outdoor temperature
was 19°C (66.2°F) and the mean temperature in closed swine
units was 7.9°C (46.2°F), PRV survived on wood alone for
16 to 19 days, while on manure contaminated wood, it sur-
vived 13 to 16 days. In the fall when the mean outdoor

temperature was 10.5°C (50.9°F) and the mean temperature
inside swine units was 4.7°C (40.4°F), the virus survived
on the wood for 20 to 21 days while on the wood contaminated
with manure it survived for 17 to 21 days. In winter and
early spring when mean temperature were -9°C (15.8°F) out-
side and 4.5°C (40.1°F) inside swine units, the virus
survived for 31 to 38 days on clean wood while on wood
contaminated with manure, it survived for 22 to 25 days. In
spring, when mean temperatures were 10°C (50°F) outside, and
15°C (59°F) inside swine units, the virus survived for 20
to 21 days on wood alone and on manure contaminated wood
for 16 to 17 days. Generally, the virus survived approxi-
mately 3 days less on the wood contaminated with manure than
on the wood alone. The humidity during these experiments
was recorded to be between 77% in the fall and 86% in the
spring. The same authors also studied the survival of PRV
on the hides of infected animals and on experimentally in-
fected hides found it to be 24-28 days in summer and 46-
49 days in winter. The virus was reported (90) to have
survived for 797 days in the fluid from a pulmonary edema
lesion stored in a refrigerator. The same authors pointed
out that PRV could survive for 30 to 50 minutes at tempera-
tures ranging between 55-60°C, for 10 minutes at 70°C, for
only 3 min. at 80°C, and that it was instantly inactivated

at 100°C. It was also reported (84) that the virus was
destroyed rapidly by boiling. In another study (50) on
the effects of temperature and urea on virulent and attenu-
ated strains of PRV, it was found that virulent strains were
resistant to 53°C temperature while the attenuated strains
were mostly sensitive.

DISINFECTANTS

A disinfectant is a chemical product that prevents infection by inactivation or inhibition of disease-producing organisms in the unanimate environment. It is desired that disinfectants function in the presence of extraneous organic matter and dense pathogenic microbial populations, regardless of the presence of saprophytic microorganisms, which may be found, that may or may not be killed (43, 59, 82, 100).

Desirable characteristics of good disinfectants have been reported to include: high germicidal efficacy with a wide spectrum of antimicrobial action lethal to bacteria, including bacterial spores, as well as to fungi, viruses and protozoa. A rapid lethal action in crevices and cavities and the penetration of organic material such as blood, sputum and feces are preferable. A disinfectant should be easily dissolved in or mix with water to form a stable liquid, non-corrosive to metal, and nondestructive to other materials (43, 59, 90, 93).

Disinfecting effectivity of any disinfectants has been reported to depend upon several factors which might be related to the disinfectants such as solubility and stability in the medium and in microbe constituents, as well as affinity for bacterial cells, quality of ionization constant,

and mode of action. Effectivity may also be related to the microorganisms to be disinfected including susceptibility of the microbes, numbers and types of contaminants, presence of microbes as vegetative or spore forms, distribution of microbes in clumps or in even suspension. Finally, effectivity may be related to the general environment which might affect the disinfection process, including temperature at which the process occurs, time intervals of exposure to disinfectants, hydrogen ion concentration or level, pressure, especially in gaseous substances, presence of organic compounds or other chemicals that inactivate the disinfectant such as blood, sputum and feces, as well as electrolytes as sodium chloride, all of which have been reported to affect the disinfection process. Esthetic factors such as odor, color, or staining quality are sometimes determinants in the choice of disinfectants. Cost is often as important consideration especially for veterinary and farm applications (43, 51, 59, 90, 93).

The mechanisms of action of disinfectants have been reported to be through different means, including oxidation of microbial cells, hydrolysis, combination with microbial proteins to form salts, coagulation of proteins, modification of the permeability of microbial plasma membranes, inactivation of vital enzymes of microorganisms and

disruption of cell structures (5, 43, 64, 78, 82, 90, 95).

Viral Disinfectants

Dunham (38) mentioned that viruses can be in-
activated by physical, chemical or biological agents.
Physical agents include moist or dry heat and irradiation.
Biological agents include body defense mechanisms.

Chemical disinfection of viruses has been found to be
related directly to the type and concentration of the viral
agents, to the presence of organic materials, to the time
of exposure to the temperature at which the disinfectant
is acting, and to the hydrogen ion concentration (2, 38,
64). Klein and Deforest (71) mentioned that the chemical
composition of the outer coats of the test viruses determines
their susceptibility to virucidal agents. The type of the
virus affects the disinfecting activity of any disinfectant
as in the case of enveloped viruses which contain lipid
constituents in their envelopes (lipophilic viruses) that
will be affected by lipid solvents and related materials (38,
64, 70). The same authors described hydrophilic unenveloped
viruses which are not sensitive to, and sometimes are
actually resistant to, lipophilic disinfectants unless
used with other chemicals which change the pH or unless the
concentration is increased to a higher level (5, 25, 38, 64,
(71). The presence of organic materials, such as pus, blood,

serum or feces greatly inhibits the action of some dis-
infectants such as surface active agents and phenolic
compounds (2, 5).

Disinfectants in Use

Kowalski and Mallmann (77) pointed out that
through the survey they conducted among 100 veterinary
practices in the state of Michigan, they found the most
commonly used disinfectant to be quaternary ammonium compound
followed by chlorhexidine. Many other workers as well as
authors, have studied or mentioned the effectiveness of
those disinfectants as well as of phenolic compounds and
sodium hydroxide against herpes viruses (4, 5, 19, 25, 27,
39, 41, 64, 80, 86, 103, 116).

Thus, due to the above mentioned comments, and as
this study dealt with one of the herpesviruses, PRV, a
lipophilic virus; the following disinfectants were chosen
for this study:

1. Chlorhexidine - Nolvasan[R]

2. Quarternary Ammonium Compound - Benzalkonium
   chloride - Roccal[R]

3. Phenolic compound - I Stroke Environ[R]

4. Alkali-Sodium hydroxide - Lye.

Chlorhexidine

Chlorhexidine is a synthetic, colorless, odorless and strongly basic compound; the chemical formula is: 1,1'-hexamethylene bis[5-(p-chlorphenyl)biguanide]. The chemical structure is:

CL-⟨◯⟩-NH- C -NH- C -NH- (CH$_2$)$_6$ -NH- C -NH- C -NH-⟨◯⟩-CL
           NH   NH   NH   NH
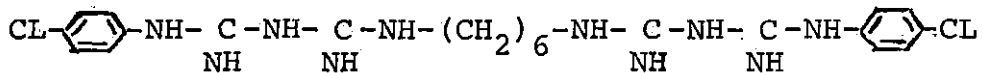
Chlorhexidine is a highly insoluble salt with a melting point of 134°. The diacetate salt solubility is far greater than the dihydrochloride salt (43, 64, 80).

Chlorhexidine has a strong alkaline reaction. It is only slightly soluble in water, so it is often formulated as a gluconate which has a good solubility in water. It is nontoxic and effective against a wide variety of Gram positive and Gram negative bacteria, and against other organisms (43, 64). It has been reported to have very little irritancy and low toxicity allowing it to be used on most sensitive mucosal surfaces (43).

Chlorhexidine has been reported to show its effect on bacteria on the cell membranes, inhibiting the adenosine triphosphate of the membrane by interfering with the net uptake of potassium ions by the intact cells. It has also been reported to disrupt the plasma membrane of

bacterial cells, even in the presence of blood (43, 59, 64, 92, 94). However, Lawrence (80) had reported that chlorhexidine is reduced somewhat in antimicrobial activity by certain organic substances, including milk, blood and serum.

It was predicted (73) that disinfectants with lipo-philic properties might inhibit the infectivity of lipid containing viruses as a result of damage of the exterior of the virions causing interference with the early stage of infection; i.e., attachment, penetration etc., and/or as a result of injury to the viral nucleic acid, prevent its complete functioning.

Chlorhexidine which has lipophilic properties (76), has been described as the most widely used of the cationic antiseptics (43) against lipophilic viruses (77). It has been found to be effective against many members of the herpesviruses; including PRV (25, 86); herpes simplex I and herpes simplex II (104); feline viral rhinotracheitis virus (100); equine infectious anemia (EIA) virus (102); and non-herpesviruses such as transmissible gastroenteritis (TGE) virus (25).

The chlorhexidine compound used in this study was Nolvasan[R] (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa). The package describes the product as follows:

Active ingredient:

1,1'-Hexamethylene bis[5-(p-chlorophenyl)biguanide]
              diacetate - 2%
Inert ingredients   -   98%

For use as a viricide and fungicide.

For use at the following concentrations:

1. "For disinfection of inanimate objects to aid in
   control of viral infection, 3 oz./gallon of clean
   water (23437.5 ppm),

2. For disinfection of veterinary or farm premises,
   1 oz./gallon of clean water (7812.5 ppm),

3. For disinfection of all meat and food establish-
   ments, after removing or protecting the food
   carefully, use at a rate of 1 oz./gallon (7812.5
   ppm) of clean water after thorough cleaning of the
   operational area."

Not effective against Pseudomonas aeruginosa or
Gram positive pyogenic cocci on inanimate surfaces.

The manufacturers directions for use of Nolvasan in

animal quarters are:

1. "Remove all animals and feeds from premises or
   from vehicles to be disinfected.

2. Remove all litter and manure from floors, walks,
   and any surface of the area to be disinfected.

3. Empty all feeding and watering appliances.

4. Saturate all surfaces with the recommended concen-
   tration of Nolvasan solution.

5. Ventilate premises or vehicles and do not return
   animals or use any equipment until the treated
   surfaces are dry.

6. All treated feeding and watering appliances must be
   scrubbed with detergents and rinsed with potable
   water prior to re-use."

The manufacturer cautions: "Keep out of reach of children. May be irritating to eyes or mucous membranes. May be harmful if swallowed. Avoid contamination of feed and feedstuffs. If in contact, flush thoroughly with water. Rinse empty containers thoroughly with water and discard."

Benzyl Ammonium Chloride

Also called Benzalkonium chloride, an alkyldimethyl-benzylammonium chloride. The structural configuration is:

$$\text{C}_6\text{H}_5 - \text{CH}_2 - \overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{N}} \overset{\oplus}{-} R \cdot CL^{\ominus}$$
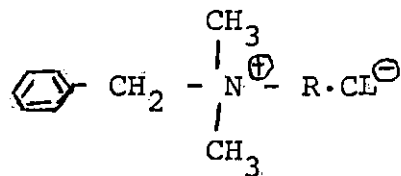
in which the R represents the alkyl radical ranging from C_8H_{17} to $C_{18}H_{37}$. It is a cationic surface active quaternary ammonium compound (51, 59, 64, 95).

Benzalkonium chloride is a white amorphous powder readily soluble in water, forming a colorless and odorless stable, nonvolatile solution, which remains so under acidic, neutral or basic conditions. An increase in temperature and of the pH of the solution will increase its effectiveness (5, 78, 94).

The surfactants, both anionic and cationic preparations, have the ability to denature proteins (5, 59, 95). The antibacterial ammonium ions derive their unique biological

activity from the long aliphatic chain present in their structure which inhibits bacterial respiration and glycolysis, as well as inhibiting certain sensitive vital enzymes (5, 95). It has also been reported that at bactericidal and bacteriostatic concentrations, they decrease the surface tension and the permeability of the plasma membrane, thus, the organism loses its ability to remain in equilibrium with its environment (64, 92, 95). The positively charged cations of quaternary ammonium compounds combine with the negatively charged anions of soap, terminating the detergent activity of each other (5, 59, 64). Thus, skin should be washed thoroughly from soap before cationic disinfectants are used (59, 64).

Cationic disinfectants combine readily with proteins, fats and some phosphates (64), thus, they are of limited value in the presence of serum, blood, and other tissue debris, and are not suitable for disinfection of premises, as the large amount of organic debris present would immediately neutralize their antibacterial activity (2, 51, 59, 64, 78, 94).

It has been reported that cationic surfactants, in general, do not possess viricidal, fungicidal or spermicidal action (64). However, quaternary ammonium compounds have been found (71) to be effective against lipophilic viruses such as herpes simplex, vaccinia and Asian influenza

viruses at a concentration of 1:10000 within 10 minutes, but not against hydrophilic viruses, polio type I, coxsackie B-1 and echo 6 even at a concentration of 1:10 for 24 hours. Activity against most lipid-containing animal viruses has been reported but not as a general viricide (77). Benzalkonium chloride at the rate of 1:1000 was found to be (38) effective against influenza and vaccinia viruses within 10 minutes; and against eastern equine encephalomyelitis virus with the presence of 1% tissue extract. In 1977, Knight (73) predicted that "viral infectivity may be lost by damage to the exterior of the virion in a manner that interferes with the early stages of infection (attachment, penetration, and so on) or by injury to the viral nucleic acid that prevents its complete functioning. Some treatments can cause both types of damage." Viruses with lipid material in their outer membranes permit penetration of the lipophilic cidal agents (5). Surfactant compounds with their chemical structure decrease the surface tension of an aqueous solution, and emulsification is augmented by altering the wettable surface and reducing the wetting angle (64). Benzalkonium chloride with its high affinity to combine with protein, fat or phosphorus (64); and its lipohilic properties (5, 71, 77), may function in these ways against herpes viruses.

Benzalkonium chloride has also been described (2) as effective against many types of viruses other than herpes viruses. The same authors reported (2) its inactivation of influenza type A, rabies, and Semliki Forest viruses. They also mentioned the sensitivity of measles and canine distemper viruses to the quaternary ammonium compounds, but vaccinia virus needed a higher concentration. The presence of serum inhibited the disinfectant action. It has also been mentioned that Zepheran chloride[R] (a quaternary compound) was effective against adenovirus type 2, vaccinia and Asian influenza viruses as well as herpes simples (71). Quaternary ammonium compounds have also been found (25) effective against transmissible gastroenteritis (TGE) virus but not against porcine parovirus (PPV).

Benzalkonium chloride may be used to preserve the sterility of surgical instruments and rubber articles during storage at a concentration of 1:1000. A 10% aqueous solution of benzalkonium chloride is commonly used for general disinfection purposes (51, 59, 64, 78, 82).

The quaternary ammonium compound used in this study was Roccal (Winthrop, Sterling Drug Inc., New York, N.Y. 10016). The package describes the product as follows:

n-alkyl dimethyl benzyl ammonium chloride - 50%
with (67% C_{12}; 25% C_{14}; 6% C_{16}, 1% C_8,C_{10},C_{18}).

The manufacturer's recommended dilution for general disinfection of floors, walls, kennels, animal cages, furniture, boats and aprons was 1:625 (1600 ppm).

According to the manufacturer's information, Roccal[R] activity has been demonstrated against canine distemper virus, canine herpes virus, and PRV at dilutions recommended for treatment of hard inanimate surfaces.

The manufacturers directions for use of Roccal are:

1. For general disinfection of hard surfaces,
   "For floors, walls, operating tables, cages and furniture in animal hospitals, kennels, animal quarters and laboratories: clean thoroughly and rinse all surface areas before disinfecting. Then apply Roccal at a dilution of 1:400 (2500 ppm) by mop sponge or spray and let air dry. For surfaces difficult to clean and heavily contaminated areas, use Roccalata dilution of 1:200 (5000 ppm)."

2. For disinfection of eating and drinking utensils for animals not considered as food sources for human consumption: "Scrape and prewash with warm water, then soak in Roccalata dilution of 1:400 (2500 ppm) for at least 10 min."

"For the preparation of the recommended dilutions: For 1:400 (2500 ppm), add 10 ml (2 teaspoons) of Roccal to 1 gallon of water. For 1:200 (5000 ppm) add 20 ml (4 teaspoons) of Roccal to 1 gallon of water. Do not mix Roccal with soap or any anionic detergent."

The warnings of the manufacturer:

"Roccal is dangerous; keep out of reach of children. It is corrosive to tissues, causes eye damage and skin irritation. Do not get in eyes, on skin or on clothing. Wear goggles or face shields and rubber gloves when handling the concentrate. Harmful or fatal if swallowed. Avoid contamination of food."

For first aid in case of contact: "Immediately flush eyes or skin with plenty of water for at least 15 minutes. For eyes, call a physician. Remove and wash contaminated clothing before reuse."

"If swallowed, drink promptly a large quantity of milk, egg whites, gelatin solution or if these are not available, drink large quantities of water. Avoid alcohol. Call a physician immediately."

Note for physician: "Probable mucosal damage may contraindicate the use of gastric lavage. Measures against circulatory shock, respiratory depression and convulsions may be needed."

Phenolic Compounds

Phenol U.S.P. is one of the oldest antiseptics, having been introduced in surgery by Sir Joseph Lister in 1867. It is also called carbolic acid, due to its weakly acidic properties. It is a monohydroxybenzen ($\bigodot^{OH}$), obtained from coal tar (48, 64).

Phenol occurs as colorless to light pink crystals or crystalline masses that have a characteristic odor. It is soluble in water (1:15), very soluble in alcohol, glycerin, fixed and volatile oils as well as in petrolatum and liquid petrolatum (1:70). Its aqueous solution gradually darkens on exposure to air and light (48, 64).

In addition to its bactericidal activity, which is not very strong, phenol has a caustic, slightly anesthetic, and in general, protoplasmic activity, being toxic to all types of cells. It causes protein precipitation under high concentration, whereas, at low concentration, it denatures protein without coagulation (48). It is also reported that under high concentration, phenol rapidly penetrates and ruptures cell walls, while in relatively low concentration, it permits the leakage of cell constituents, such as glutamic acid and other metabolites (5, 48). It has also been reported (59) that phenol is bacteriostatic in a concentration of approximately 0.2%, bactericidal above

1% and fungicidal above 1.3%.

Phenol has low affinity for protein, it does not combine firmly with the superficial proteins of cells but penetrates deeper into the tissues (59, 64, 95). Phenol action was described also as due to association with cellular lipids, because phenol has low water and high lipid solubility (5, 64, 95).

Thus, as a disinfectant with lipophilic properties, phenol might have the same mechanisms of action predicted (75) against lipid containing viruses, such as herpes viruses, by damaging the exterior of the virions and interferring with the early stages of infection; i.e., attachment and penetration. It was pointed out (5) that viruses with lipid materials in an outer membrane permit the penetration of the lipophilic cidal agents. Phenolic disinfectants may cause injury to the viral nucleic acid and prevent its complete functioning. Some treatments can cause both types of damage (73). The effectiveness of phenol against herpes simplex has been reported (71).

It has been reported that phenol is much more effective in aqueous solution than in glycerin or lipids, and it is relatively inactive when incorporated with soap (59). It has also been reported that the activity of phenol is not greatly reduced by the presence of organic matter, therefore, it is used mainly for disinfection of inanimate

objects and excreta (82, 94). The general antibacterial effects are irreversible by dilution with water, nor can bacteria affected by phenolic compounds acquire immunity to an inhibitory concentration (5).

Phenolic homologs are preparations with more lipophilic compounds to enhance the potential effectiveness of phenols. Many of the phenolic derivatives are more bactericidal than phenol itself, and the most important of these are the halogenated phenols, bisphenols, alkyl-substituted phenols and resorcinols (5, 48, 51, 59).

The phenolic disinfectant used in this study was I Stroke Environ[R] (Vestal Laboratories, Division of Chemical Corporation, St. Louis, MO 63110). It is a mixture of phenolic compounds. The package describes the product as follows:

Active ingredients:

O-phenylphenol $\overset{OH}{\underset{C_6H_5}{}}$	10.0%	(100.000 ppm)
O-benzyl-p-chlorophenol	8.5%	(85000 ppm)
p-tertiary-amylphenol	2.0%	(20000 ppm)
Inert ingredients:	79.5%	(795000 ppm)

Each of the active ingredients is more potent than phenol, but the toxicity and caustic actions are also greater (59, 82); n-amylphenol was found to gain increased effectiveness by increasing the molecular weight and it is

associated with decreasing the mammalian toxicity about 10 times less than phenol (5).

I Stroke Environ[R] at a concentration of 1/256 (3906.25 ppm) at 23°C, was found to be effective against EIA virus (102); and feline calicivirus (FCV) (100). At a concentration of 1%, 0.75% and 0.5% (10.000 ppm, 7500 ppm and 5000 ppm), I Stroke Environ was found to be effective against African swine fever within one hour at room temperature when applied in a spray form (113). It has also been effectively used in a spray form against Marek's disease virus (MDV) at a concentration of 1/250 (4000 ppm) (27). The effectiveness of I Stroke Environ in the recommended concentration (RC) against PRV and TGEV but not PPV was also reported (25).

The manufacturer's directions for use of I Stroke Environ are: "For all general cleaning and disinfection, including tuberculous areas, use 1:256 solution (1/2 oz. per gallon of water) (3906.25 ppm). I Stroke Environ is harmless to fabrics, resilient flooring, tile, terrazzo, and painted or varnished surfaces. When cleaning and disinfecting food, animal or poultry facilities, first remove all livestock from the premises, cover or remove all feed and foodstuffs, and remove all dirt, litter and debris. Then thoroughly saturate all surfaces with I Stroke Environ use solution (Rinse all surfaces contacting food, and flush

feeders and waterers with potable water after disinfecting).
Allow disinfected surfaces to dry before rehousing live-
stock."

The manufacturer warns: "Corrosive to tissues. The
concentrate causes eye and skin damage. Harmful or fatal if
swallowed. Do not get in eyes, on skin or on clothing.
Wear goggles and rubber gloves when handling. Avoid con-
tamination of food. Do not reuse empty container."

First aid: "In case of contact, immediately flush
eyes or skin with plenty of water for at least 15 minutes.
For eyes, call a physician. Remove and wash contaminated
clothing before rinse. If swallowed, do not induce vomiting,
drink large quantities of fluid and call a physician im-
mediately."

Alkalies - Sodium Hydroxide

Since antiquity, alkalies have been reported to be
used as disinfectants (64, 95).

Their mechanism of action is related to hydroxyl
ion (OH^-) concentration, and this depends on the degree
of dissociation of the drugs in use (64, 90). A pH of
greater than 9 has been reported to inhibit the growth of
most bacteria (64). The vegetative forms of most bacteria
will be killed in about 10 minutes in a 1 percent solution
of sodium or potassium hydroxide, with Gram negative forms

more susceptible than Gram positive (95).

The effectiveness of alkalies against viruses has been reported by some authors. They have been used occasionally to disinfect excreta from patients with virus infections, especially poliomyelitis (95). At 5% concentration, sodium hydroxide inactivated EIA virus slowly (102). However, Aleutian disease virus was found to be inactivated within 10 minutes exposure to 0.5% as well as to 1% sodium hydroxide (103). In two different studies, Marek's disease virus was inactivated by 3% sodium hydroxide (66), as well as by 2%, 1% and 0.5% concentrations of sodium hydroxide, but at 0.25%, it was slightly less effective in the time limit of 10 minutes (27). In another study the effectiveness of 2% sodium hydroxide (pH 12.7) against swine vesicular disease virus in hard water as well as in hard water and swine feces was described (60). The 2% sodium hydroxide was also described to inactivate the Hong Kong strain of swine vesicular disease virus within 2 minutes (20). Foot and mouth disease virus was inactivated within less than 15 seconds by sodium hydroxide at pH 12.5 at 4° and 25°C (101). For 1, 10 and 30 minutes exposure at room temperature, 4° and 37°C, Talfan virus and adenovirus were inactivated by sodium hydroxide at a final dilution of 1% (w/v) (35).

Sodium hydroxide (Lye, Soda Lye), is widely used in disinfection. Lye contains 94% sodium hydroxide, and is commonly used at a concentration of 1.88% (18.800 ppm) NaOH which has been found to be effective against a wide range of pathogens (64, 90, 95). Potassium hydroxide and calcium oxide and hydroxide have been reported to be used for disinfection by many authors as well (64, 80, 95).

Many authors in different parts of the world have reported (64) to have mixed 2% Lye (1.88%, 18.800 ppm NaOH) with many other disinfectants to increase their effectiveness. Belak and Kisary mixed 1% sodium hydroxide, 8% sodium hypochlorite, and 3-5% formaldehyde to inactivate transmissible gastroenteritis virus within 15 minutes. Walter and Coffee used an aqueous alkaline solution of hydrated lime and sodium hydroxide for control of cyrptococcus neoformans in pigeon coops (64). The same author reported that sodium hydroxide and phenol were used as disinfectant at temperatures below freezing, i.e., -5° to 0°C; and that 2% sodium hydroxide and 2% cresol were used to inactivate hog cholera virus in excreta. Sodium hydroxide and potassium permanganate were used against Talfan virus and porcine adenovirus type 2 (35). A calcium hypochlorite mixture containing 2%-3% chlorine, 2% formol, and 2% sodium hydroxide at 60° to 70°C, has been effectively

used for disinfection of transport vehicles (64).

The most commonly used alkalies which have been reported (95) are: NaOH, KOH, $NaHCO_3$, NH_3, Na_2CO_3, Na_3PO_4, $Ba(OH)_2$ and $Na_2B_4O_7 \cdot 10H_2O$. Also mentioned (64), are lime (quick lime-calcium oxide), slaked lime (calcium hydroxide), and sulfurated lime solution.

A 2% solution of sodium hydroxide was used in this study.

<div align="center">

Chemical Inactivation of
Herpesviruses

</div>

The disinfectants used in this study were proven by many workers to be effective in inactivating some members of the herpesviruses.

Chlorhexidine (Nolvasan):

Pseudorabies virus (PRV) was found to be very sensitive to chlorhexidine (Nolvasan[R]) (25); within 5 minutes the virus was completely inactivated, at the recommended 1/128 manufacturer's concentration of disinfectant. Feline viral rhinotracheitis virus was also found (100) to be inactivated by this recommended concentration of chlorhexidine within 10 minutes exposure time. Herpesvirus hominis strains were inactivated completely within 90 minutes exposure time at the recommended final dilution (200 µg/ml) chlorhexidine gluconate in PBS (4).

Quaternary ammonium compound (Roccal)

Pseudorabies virus (PRV) was inactivated within 5 minutes exposure to Roccal-D at a concentration of 1/200 (25). Fowl laryngotracheitis and herpes simplex viruses were inactivated in 10 minutes exposure time at the recommended concentration of benzalkonium chloride (Roccal or Zephiran chloride) at both a temperature of 30°C and room temperature (2). Marek's disease virus was inactivated within 10 minutes exposure to quaternary ammonium compounds (27). Only 0.13% or less infectious laryngotracheitis virus survived when exposed to quaternary ammonium compound diluted in PBS to the manufacturer's recommended dilution (39). Feline viral rhinotracheitis virus was also sensitive to the manufacturers recommended dilution of quaternary ammonium compound and was inactivated within 10 minutes exposure (100).

Synthetic phenol compound (I Stroke Environ)

Pseudorabies virus was inactivated completely at the recommended dilution (1/256) of I Stroke Environ, a synthetic phenol compound, within 5 minutes (25). Marek's disease virus was also inactivated by the same preparation at a dilution of 1:250 within 10 minutes (27). Feline viral rhinotracheitis virus was inactivated by a 1/256 concentration of I Stroke Environ within a 10 minutes exposure

time (100).

Sodium hydroxide (Lye)

Pseudorabies virus was inactivated by 5% sodium
hydroxide within 5 minutes (25). Marek's disease virus
was inactivated within 10 minutes by 2% sodium hydroxide
(27); however, in another study, 3% sodium hydroxide was
found to be ineffective in inactivating MDV (66). In-
fectious bovine rhinotracheitis virus was found to be
sensitive to 2% sodium hydroxide (41, 83).

MATERIALS

The basic requirements for virus cultivation, propagation and identification, as well as detection of the survival by plague assay techniques, depends mainly on the media and cell culture systems in use. The media, cell cultures, diluents, virus, fomites and disinfectants used in this study were the following:

Media

The propagation and cultivation media prepared in the laboratory and used in this research work had been used in related research studies (32, 44).

Minimum Essential Medium (MEM)

Minimum Essential Medium (MEM) was prepared by dissolving gently at room temperature (15-30°), dry packs of powdered media (Grand Island Biological Company, Grand Island, New York) (Table 1), in deionized triple distilled water. The appropriate salts and nonessential amino acids were contained in the prepared media packs but not sodium bicarbonate. After the dry media were dissolved, this was added at a rate of 2.2 gm of sodium bicarbonate per liter. Then, the pH was adjusted to 0.2-0.3 units lower than the desired level of 7.0 (32) using 1N sodium hydroxide or 1N hydrochloric acid. The media were then sterilized

by filtration through methyl cellulose membrane
filters (Millipore Corporation, Bedford, Massachusetts),
of 200 nm pore diameter which raised the final pH to 7.0.

To control any contamination that might result in
bacterial or fungal growth in the media or cell cultures,
antibiotics were added as shown in Table 2. The final
solutions were checked by plate inoculation for bacterial
sterility.

Minimum Essential Medium (MEM), prepared in single and
double strength, was stored at 4°C, from which the fol-
lowing were prepared.

Growth Medium (GM)

Growth Medium (GM) was prepared from single strength
MEM by adding 10% fetal bovine serum (FBS) (Sterile
Systems Inc., Logan, Utah), to the required quantity of MEM
and also antibiotics if not added to the stock.

Maintenance Medium (MM)

Maintenance Medium (MM) was also prepared from the
single strength MEM by the addition of 2% (FBS) to the
required quantity of MEM, as well as antibiotics if not
added to the stock.

Overlay Medium

Overlay Medium was prepared from double strength MEM with an equal quantity of gum tragacanth solution (described below) and 2% FBS, as well as antibiotics if not added to the stock MEM.

Gum Tragancanth Solution

Gum Tragacanth Solution was prepared by adding slowly 1.6 grams of gum tragacanth (Fisher Scientific Company, Pittsburgh, PA) to 100 ml of warm triple distilled water with agitation, and sterilized by autoclaving for 30 minutes at 121°C.

Diluents

The diluents used throughout the different experiments in this study (listed in Table 6-B) included the following:

Saline G

Saline G constituted the main virus diluent for comparative tests in this study. It had been used previously (13, 32, 33, 44). Table 3 shows the complete formula of saline G. The ingredients were dissolved gently at room temperature; pH was adjusted to 0.2-0.3 higher than the required level and sterilization was performed by filtration

through a 200 nm pore diameter filter (Millipore Corporation, Bedford, Massachusetts). The pH was then corrected by adding 0.1N sodium hydroxide or 0.1N hydrochloric acid to reach the required pH 7 before using the solution in this study.

Physiological Saline Solution (PSS)

Physiological Saline Solution (PSS) was prepared by dissolving 8.5 grams of sodium chloride in one liter of distilled water. Sterilization was by autoclaving for 15 minutes at 121°C. The pH was then adjusted to between 6.9-7.1 with 0.1N sodium hydroxide or 0.1N hydrochloric acid.

Phosphate Buffer Solution (PBS)

Phosphate Buffer Solution (PBS) was used in some experiments in this study. Table 4 shows the full formula. The materials were dissolved in triple distilled water and sterilized by autoclaving for 20 minutes at 121°C. PBS was prepared in single strength and ten times strength.

Distilled water

Distilled water was used to dilute the disinfectants to double or ten times the strength (2X or 10X) of the manufacturers' recommended concentration, and was used in the preparation of different other diluents in this study

as well.

Trypsin-Versene solution

Trypsin-Versene solution was used to remove the cell
monolayers from the substrate. The complete formula is
shown in Table 5. The solution was prepared by mixing
the constituents other than trypsin gently in 180 ml of
triple distilled water, heating the mixture to 37°C
then adding the trypsin over approximately one hour with
continuous mixing to dissolve completely. Triple distilled
water was then added to make 200 ml and the solution was
sterilized by filtration through a methyl cellulose filter
(Millipore Corporation, Bedford, Massachusetts), of 200 nm
pore diameter. This resulted in ten times the required
concentration of trypsin-versine solution and it was
diluted 1:10 in triple distilled water before use.

Saliva

Saliva was collected from 12, 3-4-week-old pigs
tested free of PR. The pigs had been injected with one
injection of 1% Pilocarpine hydrochloride intramuscularly
(IM) each. The drooling saliva was collected in clean
sterile beakers and stored at 4°C overnight to allow
settling down of all debris and foreign ingredients sus-
pended in the saliva. On the next day, the clear super-
natant portion was aspirated and poured into sterile 50 ml

bottles and stored at -20°C.

Nasal washings

Nasal washings were collected from the nostrils of
pigs tested free of PR, refrigerated and cleared of any
suspended material that might be present, placed in bottles
of 50 ml each and stored until used at -20°C.

Cell Cultures

The cell line used in this study was of Madin-Darby
bovine kidney (MDBK) cells, the true passage level of which
is not known. The same cell line was used in other studies
in this laboratory (13, 32, 33, 34, 44).

Single-use plastic 250 ml tissue culture flasks
(Corning Glass Works, Corning, New York), were used to
grow the cell monolayers, using GM with antibiotics.
The cultures were incubated at 37°C in a humid chamber
with 5% carbon dioxide atmosphere with the screw caps
loosened slightly to allow gaseous exchange.

After the cells had grown into full confluent mono-
layers, usually within 48-72 hours, the GM was poured off
and the cells were removed from the flasks using trypsin-
versene solution. Suspended cells were passaged into new
flasks for another passage or into single-use eight-well
plastic plates (Luxplate, Lux Scientific Corporation,

Newbury Park, California), for experimental use. They were
incubated in the same environment mentioned above.

Virus

An eighth laboratory passage of PRV "Iowa strain" or
"Wilson strain" S62/26 which was collected by G. W.
Beran (Department of Veterinary Microbiology and Preventive
Medicine, College of Veterinary Medicine, Iowa State
University, Ames, Iowa) in 1974, and which was subjectively
described as moderately virulent, was used throughout this
study. The virus passage techniques were described by
Davies (32) and the aliquots were separated in vials
of 1, 3 and 5 mls each and stored at -90°C.

Fomites

The fomites included in this study are listed in
Table 6a. They were selected as the most probable
materials that may contribute to the transmission of the
virus from infected to susceptible animals through the
environment, and which may be chemically disinfected with-
out causing any harm to the animals. PRV had been found to
survive for variable periods on the same fomites by
Freund (44) as well as other investigators discussed in
the literature review.

Disinfectants

The disinfectants listed in Table 7 were chosen for this study primarily because they represented the active ingredients of most commercially available disinfectants. Secondly, they are widely used in laboratories and veterinary clinics as well as on farms. Thirdly, their effectiveness against enveloped viruses in general, and especially herpes viruses, has been well-documented.

The concentrations of the disinfectants used in these experiments were serial dilutions from the manufacturer's recommended concentrations for field use (FCFU). The disinfectants were first diluted to 10X or 2X the RCFU with distilled water and then to the test dilutions with the virus-diluent-fomite mixture used in the tests.

Table 1. Minimum Essential Medium (MEM) composition[a]

Component	Ingredients	Weight mg/L
a. Salts and sugar components		
Earle's salts	Calcium chloride anhyd. (CaCl$_2$)	200.00
	Potassium chloride (KCl)	400.00
	Magnesium sulfate anhyd. (MgSO$_4$)	97.67
	Sodium chloride (NaCl)	6800.00
	Sodium phosphate.Dihydrogen (monobasic) (NaH$_2$PO$_4 \cdot$H$_2$O)	140.00
Sugar	Glucose	1000.00
Phenol red		10.00

Amino acids		Vitamins	Weight mg/L
b. Amino acids and vitamins			
L-Alanine	8.90	D-Calcium pantothenate	1.00
L-Arginine·HCl	126.00	Choline chloride	1.00
L-Asparagine.H$_2$O	15.00	Folic acid	1.00
L-Aspartic acid	13.30	I-Inositol	2.00
L-Cystine.2HCl	31.29	Nicotinamide	1.00
L-Glutamic acid	14.70	Pyridoxal HCl	1.00
L-Glutamine	292.00	Riboflavin	0.10
Glycine	7.50	Thiamine HCl	1.00
L-Histidine HCl.H$_2$O	42.00		
L-Isoleucine	52.00		
L-Leucine	52.00		
L-Lysine HCl	72.00		
L-Methionine	15.00		
L-Phenylalanine	32.00		
L-Proline	11.50		
L-Serine	10.50		
L-Threonine	48.00		
L-Tryptophane	10.00		
L-Tyrosine (Disodium salt)	52.10		
L-Valine	46.00		

[a]Gibco, Grand Island Biological Company, Grand Island, New York 14072. Available in appropriate packs of pre-mixed media along with L-glutamine, nonessential amino acids and some vitamins (Table 1-b) but without sodium bicarbonate.

Table 1 (Continued)

Component	Composition	Comments
Minimum Essential Medium Cat. #410-1500	Earle's salts L-Glutamine Non-Essential Amino Amino Acids	1 pack of 96.1 gm
Deionized distilled water	4750 ml	Dissolve by gentle stirring at room temp.
Sodium bicarbonate	22 gm ($NaHCO_3$)	2.2 gm/lt. with continuous stirring
Deionized distilled water	Add to make 5000 ml	
pH adjustment	use 1N NaOH or 1N HCl	Adjust pH to 0.2-0.3 below desired level[b]
Sterilization	Filtration through methyl cellulose filter	Use 200 nm pore filter[c]
Result	Double strength MEM	Store at 4°C
To use	dilute 1:2 with deionized distilled water	

[b]pH will rise 0.1-0.3 after filtration.

[c]Millipore Corporation, Bedford, Massachusetts.

Table 2. Antibiotics used in Minimum Essential Medium

Pharmaceutical name	Trade name	Producer	Quantity used
Penicillin-G	Crystalline Penicillin G-potassium	E.R. Squibb & Co. Inc., Princeton, NJ 08540	100 units/ml
Gentamicin Sulfate	Gentocin[R]	Schering Corp., Kenilworth, NJ 07033	50 µg/ml
Amphotericin B	Fungizone[R]	E. R. Squibb & Co, Inc., Princeton, NJ 08540	3.3 µg/ml
Streptomycin Sulfate	Streptomycin	Pfizer Inc. New York, NY 10017	100 µg/ml

Table 3. Preparation of Saline G

Components	Quantity	Comments
Dextrose	1.1 grams	
Sodium chloride (NaCl)	8.0 grams	
Potassium chloride (KCl)	0.4 grams	
Sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$)	0.29 grams	
Potassium phosphate (KH_2PO_4)	0.15 grams	
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	0.016 grams	
Lactalbumin hydrolysate	1.0 grams	
Phenol red	0.0012 grams	
Triple distilled water (deionized)	add to 1 liter	Gentle stirring, dissolve at room temperature

Adjust pH level to 0.2-0.3 below desired level using 1N sodium hydroxide or 1N hydrochloric acid. pH will rise after filtration by 0.1-0.3.

Sterilize solution by filtration through a methyl cellulose filter of 200 nm pore diameter.

Store at 4°C

Table 4. ·Composition and preparation of Phosphate Buffered Saline Solution (PBS)

Materials	Quantity	Comments and Methodology
Sodium phosphate (Na_2HPO_4)	4.60 gm	(0.008 M)
Sodium thiophosphate ($NaH_2PO_4H_2O$)	1.10 gm	(0.002 M)
Sodium chloride	34.0 gm	
Deionized distilled water	4 liters	Dissolve by gentle continuous stirring at room temperature

Sterilize by autoclaving at 121°C for 20 minutes. For pH adjustment, use 1N sodium hydroxide or 1N hydrochloric acid to 7.0-7.3

Table 5. Composition and preparation of trypsin-versene solution

Component	Quantity	Comments
Dextrose	2.0 grams	
Sodium chloride (NaCl)	16.0 grams	
Potassium chloride (KCl)	0.8 grams	
Sodium bicarbonate (NaHCO$_3$)	1.4 grams	
Versene[a]	0.4 grams	
Phenol red 1% solution	2.0 ml	
Deionized triple distilled water	180.0 ml	Mix and dissolve with gentle stirring at room temperature. Then heat to 37°C before the trypsin addition.
Trypsin[b] 1:250	1 gram	From hog pancreas, porcine parvovirus free. Mix with continuous stirring until trypsin is dissolved completely, approximately 1 hour

Add deionized triple distilled water to make   200 ml

Sterilize by filtration through 200 nm pore diameter methyl cellulose filter[c]. Result is 10X the needed concentration of trypsin-versene solution. To use, dilute 1:10 in sterile deionized triple distilled water

[a]Disodium ethylene diamine tetracetate, Nutritional Biochemical Corp., Cleveland, Ohio.

[b]Gibco, Grand Island Biological Company, Grand Island, New York.

[c]Millipore Corporation, Bedford, Massachusetts.

Table 6a. The fomites and their sources used in study
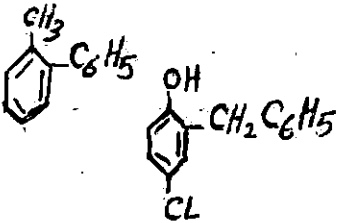
Fomite	Source
Steel	Stainless steel bucket
Concrete	Concrete flooring
Wood	Wood bar and swine bedding
Soiled bedding	Animal house-husbandry area
Loam soil	Ames, Iowa
Polypropylene plastic	Plastic milk jug
Vinyl rubber	Rubber boots

Table 6b. The diluents used in this study

Diluents	Source
Saline-G	Laboratory prepared
Physiological Saline Solution (PSS)	Laboratory prepared
Saliva	Pseudorabies virus free young pigs
Nasal washings	Pseudorabies virus free young pigs

Table 7. The disinfectants used in this study

Pharmaceutical name	Trade name	Chemical composition
chlorhexidine	Nolvasan[R]	1,'1-Hemxamethylene bis[5(p-chlorophenyl)biguanide] diacetate - 2% Inert ingredients - 98%
Quaternary	Roccal[R]	n-alkyldimethylbenzyl ammonium chlorides - 50% (C_{12} 67%, C_{14} 25%, C_{16} 7%, C_8, C_{10}, C_{18} 1%)
Phenolic compound	1 Stroke Environ[R]	O-phenylphenol - 10% O-benzyl-p-chlorophenol - 8.5% p-tertiary-amylphenol - 2.0%
Alkali	Lye	Sodium hydroxide

Chemical composition	Manufacturer	Recommended concentration (ppm)	
CL-〈 〉-NH- C -NH- C -NH-(CH$_2$)$_6$- (with NH below each C) NH- C -NH- C -NH-〈 〉-CL (with NH below each C)	Ft. Dodge Laboratories, Fort Dodge, Iowa	3 oz/ gallon	23437.5 ppm
〈 〉-CH$_2$-N$^{\oplus}$(CH$_3$)(CH$_3$)-R·CL$^{\ominus}$	Sterling Winthrop, Inc., NY, NY	1/625	1600 ppm
CH$_3$ / C$_6$H$_5$ ... OH ... CH$_2$C$_6$H$_5$ ... CL	Vestal Labora- tories, St. Louis, MO 63110	1/256	3906.25 ppm
NaOH	Many	2%	21300 ppm

METHODS

Cell Culture Preparations

After confluent monolayers of MDBK cells were grown in 250 ml flasks (Corning Glass Works, Corning, New York), the supernatant GM was decanted and the cells removed with sufficient quantieis of 1X trypsin-versene solution (7-10 ml). The cell-trypsin-versene mixtures were then transferred to 50 ml conical plastic centrifuge tubes, and centrifuged at 600 revolutions per minute (rpm) for 10 minutes. The cells settled down as pellets and the trypsin-versene solution was decanted and discarded. The cell pellets were resuspended again in centrifuge tubes in 20 ml fresh GM with gentle mixing so as not to harm the cells, then transferred to sufficient quantities of GM to make dilutions three times greater than hitherto.

From these last cell suspensions, the required number of single use eight-well plates (Lux Plates, Lux Scientific Corporation, Newbury Park, California), were inoculated at the rate of 2 ml per well, i.e., 16 ml per single plate. The next passage of the cells was commonly made by inoculating new 250 ml flasks with 20 ml of the same cell suspensions.

The plates and flasks were incubated in a humid chamber at 37°C with 5% carbon dioxide atmosphere with the screw caps kept loosened to maintain gaseous exchange. Confluent layers

of MDBK cells were usually grown up within 36-48 hours.

Fomite Preparations

All the fomites used, as shown in Table 6a, were filed or cut into small pieces of 1-2 mm^2 as in the case of steel, plastic or rubber, ground into fine particles as in the case of concrete or soil, or used in natural condition as in the case of soiled bedding which could easily be mixed into solution. These preparations helped make accurate measurements of the fomite materials possible through the experiments, made possible the placing of them in maximum contact with the virus-diluent-disinfectant combinations, made possible the keeping of the entire system at desired moisture-temperature-pH levels, and permitted sampling as desired.

Disinfectant Preparations

All the disinfectants were diluted with distilled water to 10X and 2X the RCFU, which were then diluted in the mixture with the virus-diluent-fomite mixtures and tested at 0.001, 0.01, 0.1 and 1X of the RCFU.

Diluents

Saliva and nasal washings

Saliva and nasal washings were stored at -20°C; then 24 hours before an experiment, the needed quantity was removed from -20°C to 4°C and then to 25°C or 4°C, the working temperatures in a water bath or ice bath, respectively.

Saline G, PSS, distilled water and PBS

These were kept at 4°C and warmed to 25°C in a water bath before the start of experiments, or maintained at 4°C in an ice bath.

Virus

The vials containing the virus aliquots were kept at -90°C. Just prior to use, the needed vials were thawed to 4° or 25°C to minimize virion damage and loss of titers.

Virus Titration

Virus dilutions

Sets of 6 numbered 126 x 16 millimeter test tubes were placed in racks in an ice bath, each containing 4.5 ml of saline G. Then 0.5 ml of freshly thawed stock virus was transferred to the first test tube with a sterile 1 ml pipette. Another sterile pipette was used to thoroughly mix the virus-saline G in the first test tube to ensure the even

distribution of the virions within the suspension. Then 0.5 ml was transferred to the second test tube. Further, serial 10-fold dilutions were made in the same manner, preparing $10^{-1} \div 10^{-6}$ dilutions of the stock virus.

Virus titration

Titration of the virus was performed by inoculating 1.0 ml of each virus dilution into each of 2 cell culture wells, incubating 48 hours and counting the plaques. Experimental tests were performed using 10^{-2} final dilution of the stock virus throughout.

Cell Culture Inoculation

Confluent monolayers of MDBK cells between 36 and 48 hours after seeding in eight-well plastic plates were assigned randomly to each of the decimal virus dilutions, using 2 replicate wells per dilution. Two control wells were also used for each component of the tests; i.e., virus, fomites, diluents and growth and overlay media.

The GM covering the cells was aspirated and one ml from each dilution, starting with the highest dilution, was inoculated into each of the two test wells. The plates were then incubated for one hour adsorption time in a humid chamber at 37°C with 5% carbon dioxide atmosphere. After the adsorption, the inocula, diluents and GM were aspirated

from the inoculated wells and replaced with overlay media.
The plates were reincubated in the humid chamber for another
48 hours for virus replication in the infected cells and
formation of plaques.

Fixation

The plates were taken out of the humid chamber to
room temperature and the cell layers were fixed with 2 ml per
well of approximately 6% weight/volume of formalin solution
for 20 minutes.

Staining

The media-formalin mixtures were discarded and the cell
layers were stained with 1-2 ml of 2% crystal violet aqueous
solution for ten minutes. Then the crystal violet was dis-
carded and the plates were washed under tap water and air
dried. The plaques in the countable wells were counted,
averaged and multiplied by the virus dilution to calculate
the titer of active pseudorabies in the inoculum.

Virus Assay Techniques

The assessment of disinfectant, fomite, diluent and
temperature effects on the pseudorabies virus (PRV), as
well as titrations and identification by serum naturalization
tests was performed by plaque assay technique for active
virus in the cell cultures (MDBK cells). These plaques

were formed as the virus was replicated in the infected cells, causing a cpe with cell destruction and formation of diagnostic plaques of 1.5-2 mm diameter.

The Effects of Test Diluents

Four sets of six, 126 x 16 millimeter test tubes were placed in racks in an ice or water bath at either 4°C or 25°C, respectively. Each set was assigned randomly to one of the test diluents listed in Table 6b. From each diluent, 4.5 ml were placed into first test tube of the assigned set. Then, 0.5 ml of the PRV suspension was added to the first tube of each dilution. Progressive 0.5 ml transfers produced serial 10-fold dilutions of the virus in each diluent. The serial dilutions were then assayed on MDBK cell layers as described for the virus assay technique. The pH was monitored using a Chem-Mat (Beckman Instruments, Inc., Fullterton, CA 92634), pH meter.

The Effect of the Combined Fomites
and Diluents

The combined effects of the fomites and diluents against PRV were investigated in these experiments to assess their actions.

Samples of 0.5 gm each of the test fomites listed in Table 6a were added to 4.0 ml of each of the four test

diluents listed in Table 6b in 126 x 16 millimeter sterile
glass test tubes. Then, the test tubes were placed in racks
in either an ice bath (4°C) or water bath (25°C). A vial
of the stock virus was thawed and diluted 1:10 in saline G,
making the virus working solution. Then 0.5 ml of this virus
working solution was added to each fomite-diluent test tube,
resulting in a ratio of 1:1:8 virus-fomite-diluent, respective-
ly. The mixtures were agitated thoroughly using hand shaking,
mechanical shaking or Vortex (Scientific Products, American
Hospital Supply Corporation, McGow Park, Ill. 60085), shaking
for one minute.

After three minutes reaction time, the test tubes con-
taining the reacted mixtures were centrifuged for 2 minutes
at 1000 rpm. The solid materials were sedimented and 0.5
ml from the clear supernatant solutions was transferred
to 4.5 ml PSS diluent at the same temperature, and after
thorough mixing 0.5 ml was transferred from this to another
test tube, resulting in two decimal dilutions of the reacted
mixtures.

From each test tube, i.e., undiluted supernatant, 1:10
and 1:100 dilutions, 1.0 ml samples were inoculated on random
bases per well on the monolayer of MDBK cells to assay for
remaining active virus as described earlier. Wells with
not over 600 countable plaques at the lowest inoculated

dilution were used when possible in reading the results.

The Effects of the Test
Disinfectants

Effect of the test disinfectants on the cell culture

Stock solutions of each disinfectant were made at 10 times the RCFU in distilled water (Table 7). Working dilutions were made at 1X and 0.1, 0.01 and 0.001 of the RCFU in PSS. Samples of 1.0 ml of each dilution were inoculated on MDBK cell monolayers. After one hour adsorption time, the inocula were aspirated and replaced with 2 ml per well of overlay medium and the plates were incubated in a humid chamber at 37°C with 5% carbon dioxide atmosphere. After 48 hours, the cell layers were fixed, stained and examined for levels of cell injury by the test disinfectants.

Effects of the combined disinfectants, fomites and diluents on pseudorabies virus

Preparation of disinfectant solutions   The stock 10X or 2X dilutions based on the RCFU of the disinfectants were diluted to final dilutions of 1X, 0.1, 0.01 and 0.001 of the RCFU in test mixtures with virus; fomites and diluents.

Preparation of the virus test suspensions   For each
experiment, one vial of stock PRV was thawed and diluted to
10^{-1} in saline G. This was diluted to 10^{-2} final dilution
in the test mixtures with disinfectants, fomites and
diluents.

Preparation of the fomite test suspensions   Powdered,
ground, cut or unprocessed fomites were mixed in 0.5 gm
samples with 4.5 ml of test virus-disinfectant-diluent
mixtures to give a final 10^{-1} w/v suspension.

Preparation of the diluent suspensions   Diluents were
placed in 3.5 ml aliquot in the test tubes for each experi-
ment. The addition of virus, disinfectants and fomites
gave final test suspensions of 1:1:1:7 of virus, disin-
fectants, fomites and diluents, respectively.

Reaction of the test combinations   The order of
placing the test components was initially the diluents, then
the fomites and then either virus followed by disinfectants
or in most experiments, disinfectants followed by virus.
Pipet mixing was used, followed by 3 or 28 minutes reaction
time and 2 minutes further reaction time during centrifugation.

<u>Preparation of the post-reaction dilutions</u>   Immediate-ly following centrifugation, 1:10 and 1:100 dilutions were made in physiological saline solution, always transferring 0.5 ml into 4.5 ml. Cell cultures were inoculated, placing 1.0 ml from each of the original reacted mixtures and the 1:10 and 1:100 dilutions on each of 2 wells of the cell culture plates. Following 1 hour adsorption time in a humid chamber at 37°C with 5% carbon dioxide atmosphere, the inocula were removed and replaced with overlay medium. The cell monolayers were then incubated under the same conditions for 48 hours, fixed and stained as discussed above. Plates to be read were selected on the bases of the lowest post-reaction dilutions; i.e., original reacted mixture, 1:10 or 1:100 which was not cytotoxic and in which the highest number of plaques was below 600 per well. In most tests, this was the 1:100 dilution but in some it was the 1:10 or the original reacted mixture.

RESULTS

Effects of Environmental Factors
on PRV Survival

PRV survival outside the living host, as studied in 1979 by Davies (32), was found to be temperature and pH dependent (Figure 1). The virus was most stable at pH 6.2-7.7, and even at room temperature stayed at an infectious level more than 22 days. However, at extreme levels of pH 4.3 or 9.7, the virus was highly unstable. At 4°C, the virus was stable at levels of pH 6.2-8.8 for more than 2 months before it dropped below the infectious level.

Fomites common to hog raising operations, as well as liquids, including liquid wastes that might be contaminated with PRV, were found to affect the virus survival (Table 8), and might give support or inhibition for an extended period of time.

Pseudorabies virus (PRV) was suspended in PSS diluent at 25°C and mixed by pipet, hand or Vortex^R shaking for 5 minutes with the fomites used in this study (Figure 2). The virus survival was found to be greatly affected by pH levels and intensity of contact. With the first two methods of shaking, there were no significant differences in virus titer between solutions without fomites (control) and those with fomites other than concrete. In the presence of PSS,

Figure 1.   Effects of environmental factors (pH and temperature) on PRV
             survival, using saline G diluent at 4° and 25°C and pipet
             mixing
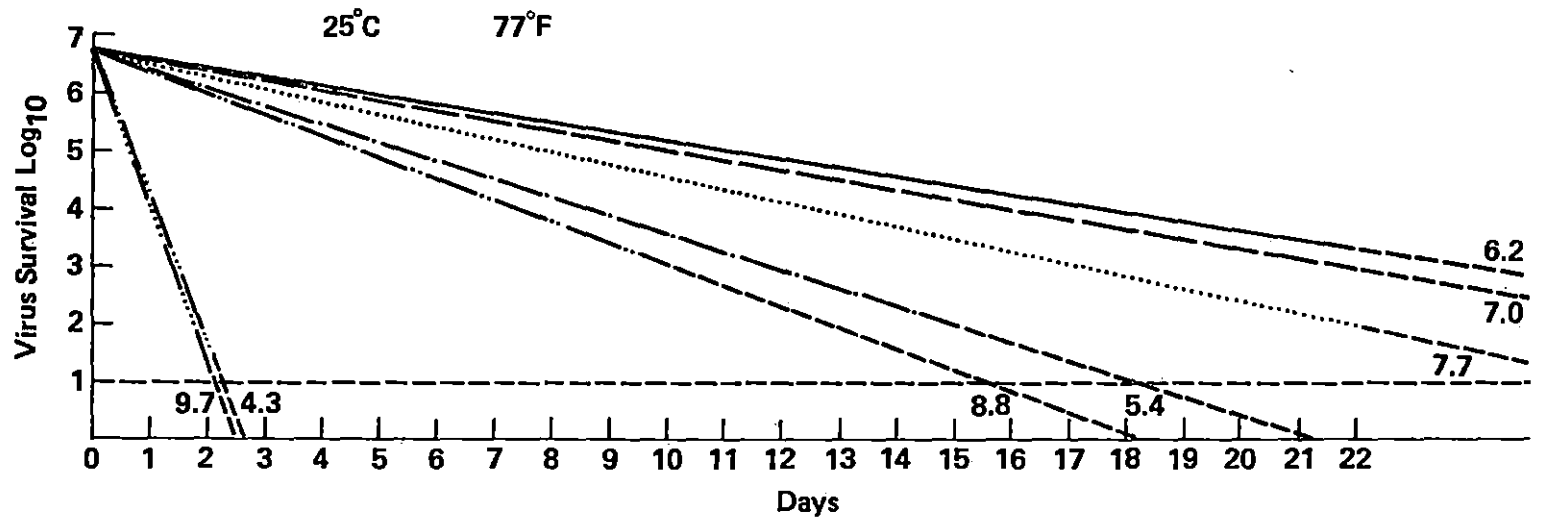
Fig. (1-a)
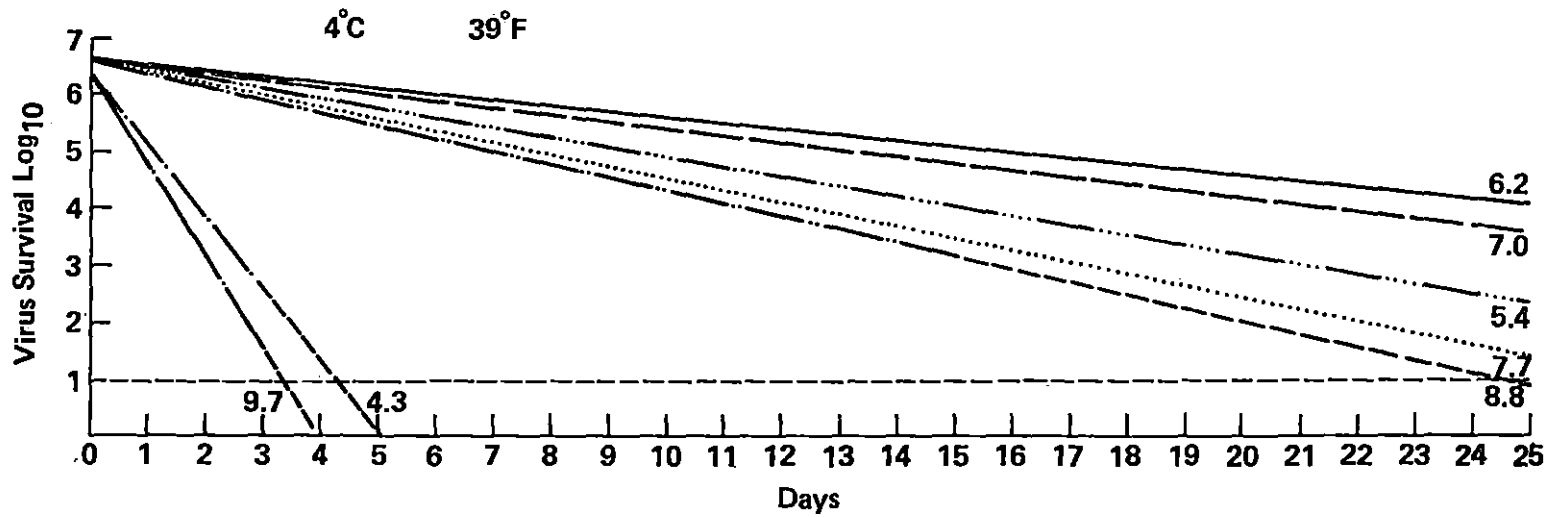
Fig. (1-b)

Table 8. Survival of PRV in contact with selected fomites in three suspending fluids, 25°C, pipet mixing, minimum infective titer 10^{-1} $TCID_{50}$/ml (Freund and Beran, 1980)

Fomite	Survival at infectious level in days			
	In saline G	In saliva	In nasal washing	Maximum survival
Concrete	<1	4	2	4
Rubber	7	2	2	7
Sawdust bedding	<1	2	<1	2
Plastic	8	3	3	8
Straw bedding	3	4	4	4
Loam soil	4	7	2	7
Steel	18	4	2	18
Denim cloth	<1	<1	<1	<1
Green grass	<1	2	<1	2
Shelled corn	36	4	2	36
Pelleted hog feed	3	3	3	3
Meat and bone meal	5	2	2	5
Alfalfa hay	<1	<1	<1	<1
Feces	2	2	2	2

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Figure 2. Effects of contact intensity with each of the
            fomites tested on PRV survival, using PSS
            diluent at 25°C for 5 minute virus contact

Fig. (2)

Legend: Pipet Mixing, Hand Shaking, Vortex Shaking

2-a NO FOMITE: pH 7.1, pH 7.1, pH 7.2

2-b CONCRETE: pH 11.6, pH 11.8, pH 12.2

2-c CONCRETE + pH BUFFER: pH 7.8, pH 8.0, pH 9.1

2-d CONCRETE + HCl ADJUSTED: pH 8.4, pH 8.9, pH 9.4

2-e RUBBER: pH 7.3, pH 7.3, pH 7.5

2-f OLD WOOD: pH 6.8, pH 6.9, pH 6.7

2-g NEW WOOD: pH 5.4, pH 5.2, pH 4.9

2-h PLASTIC GRATE: pH 7.3, pH 7.4, pH 7.8

2-i BEDDING: pH 6.9, pH 6.6, pH 6.8

2-j SOIL: pH 6.9, pH 7.2, pH 7.1
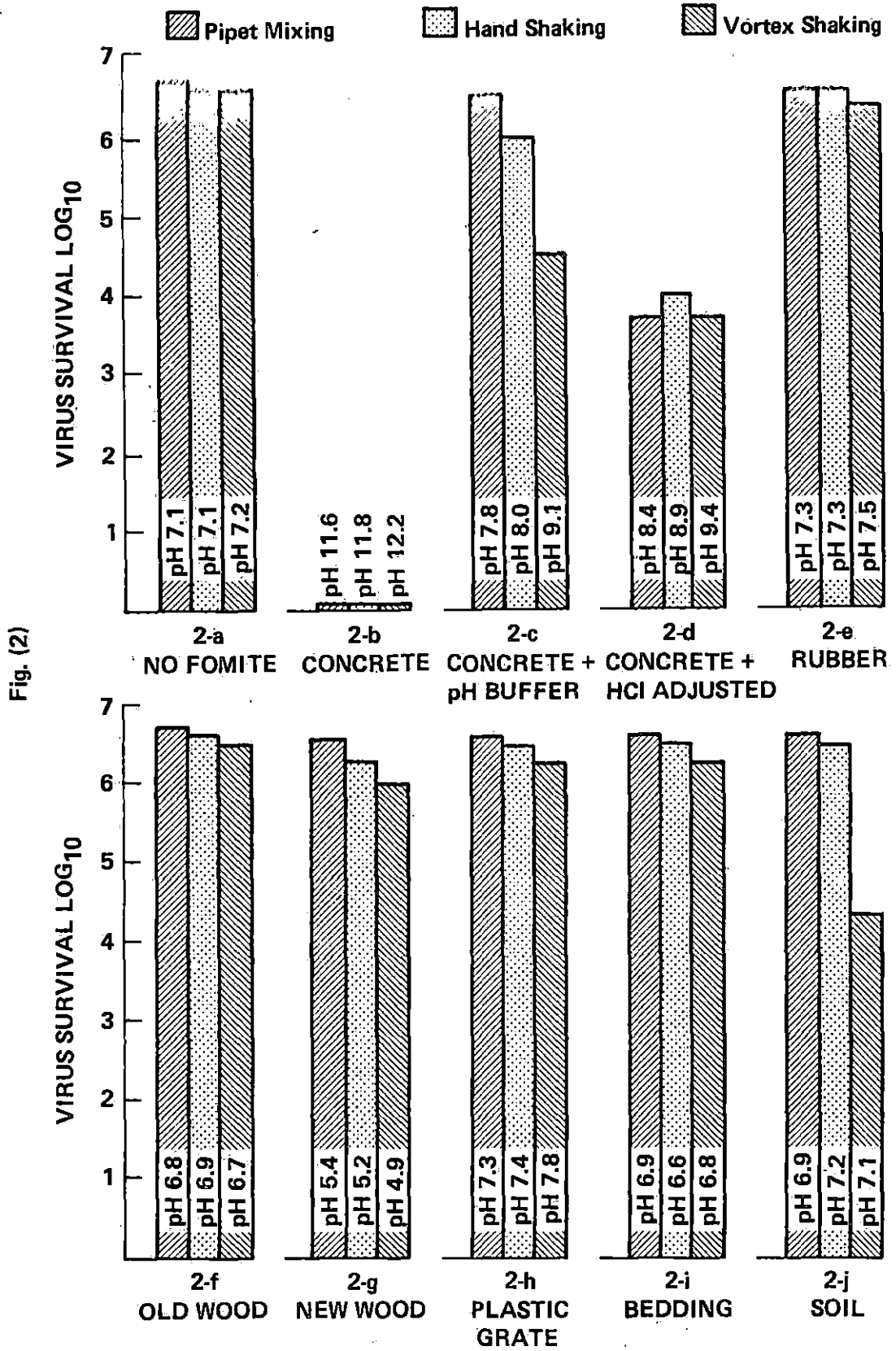
concrete had generated a high alkaline pH (11.6-12.2) which

inactivated the virus within 5 minutes with any method of

mixing. With 10X concentration of PBS solution, in contact

with the concrete, the pH was brought down to 8.0 ± 0.2

resulting in no detectable depression in virus titer within

the time of the test as compared to the control solution by

pipet mixing; and only ≈ 0.5 log by hand shaking. Hydro-

chloric acid was used to lower the pH of PSS in contact

with concrete to pH 7.4, but pipet, hand and vortex shaking

raised it again to 8.4, 8.9 and 9.4, respectively, resulting

in significant depression of the virus titer. Vortex[R]

shaking decreased the PRV titer with some fomites, by

lowering the pH of PSS in contact with new wood to 4.9 and

by increasing contact of virus with loam soil particles.

Fomite-diluent effects on PRV survival within one

hour contact as compared with no fomite (Figure 3), were

studied at 25°C, using pipet mixing. As PSS contains no

buffer activity, the virus was subjected to the direct

effects of the fomites, resulting in instant inactivation

with concrete, sharp decline in virus titer with new wood,

and significant variability in titer with some other fomites.

Saline G which has minimal buffer activity, provided some

virus protection; it did not compensate for the alkalinity

caused by concrete; it decreased the new wood effect, and the

Figure 3. Effect of contact time on PRV suspended in each
of the four diluents tested with no fomite
and six fomites at 25°C using pipet mixing
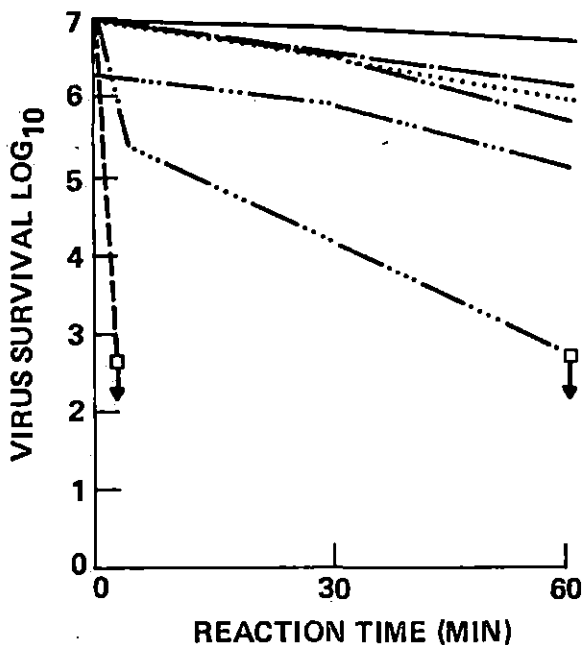
Fig. (3-a) PSS
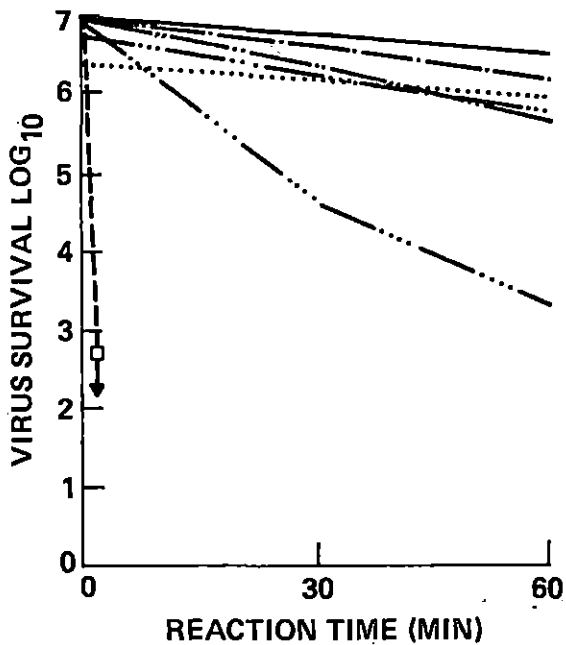
Fig. (3-b) SALINE G
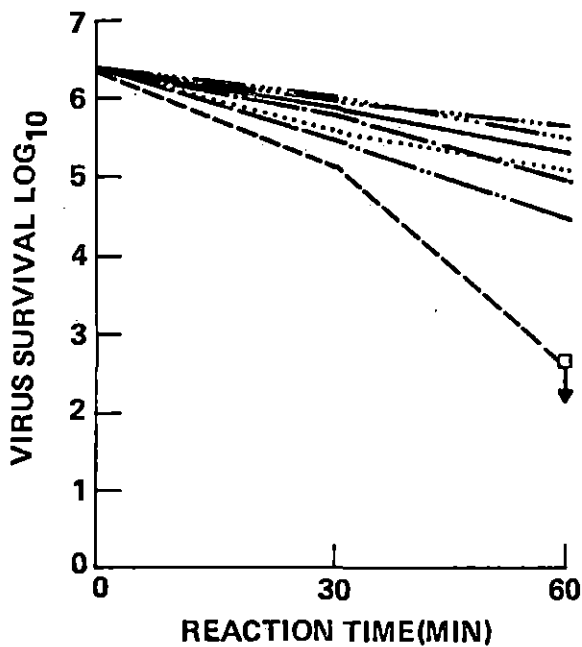
Fig. (3-c) SALIVA
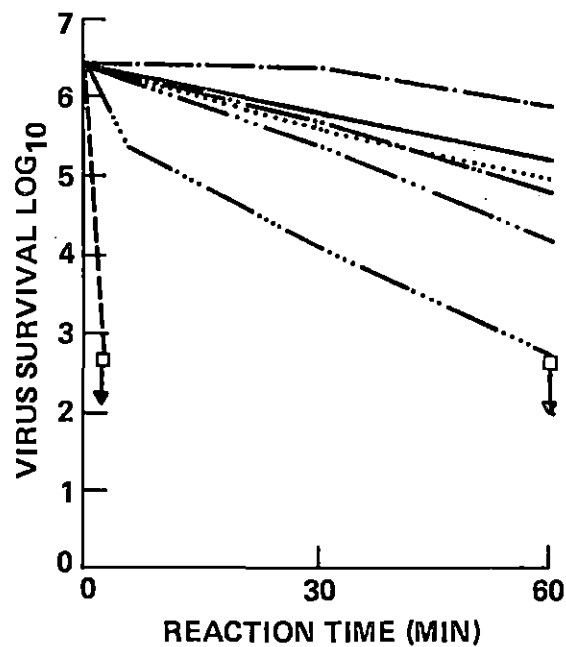
Fig. (3-d) NASAL WASHING

variability from contact with other fomites. Saliva without fomite contact showed some inactivating activity, but was found to be the most protective diluent for the virus from the effects of concrete and new wood as well as other fomites. The virus suspended in saliva in contact with new wood and loam soil survived better than in saliva alone. Nasal washing also showed inactivating activity to PRV in the absence of any fomite, and as with PSS, provided no protection for the virus from the action of concrete, resulting in instant inactivation of the virus. In contact with new wood or other fomites, the virus in nasal washings showed significant variability in its survival. However, in the presence of soiled bedding, the virus survived better than in nasal washing alone.

The high level of alkaline pH (11.8 $\pm$ 0.4) caused by concrete fomite was found to be difficult to change. Repeated washings of weathered concrete with PSS diluent and vortex shaking for various lengths of time intervals (Figure 4) dropped the pH slightly when the PSS was repeatedly changed. However, as the period of contact between washings was extended, the pH was increased and maintained at high level for a long time. Thus, as seen from Figures 2, 3, and 4, clean concrete strongly inactivated PRV by the high alkaline pH exerted by this fomite.

Figure 4. Effect of washing of concrete on pH of
          washing fluids. PSS washing fluid, 25°C,
          Vortex shaking arrows (↑) indicate times at
          which washing fluid was removed and re-
          plenished with fresh fluid

WASHING TIME IN MINUTES
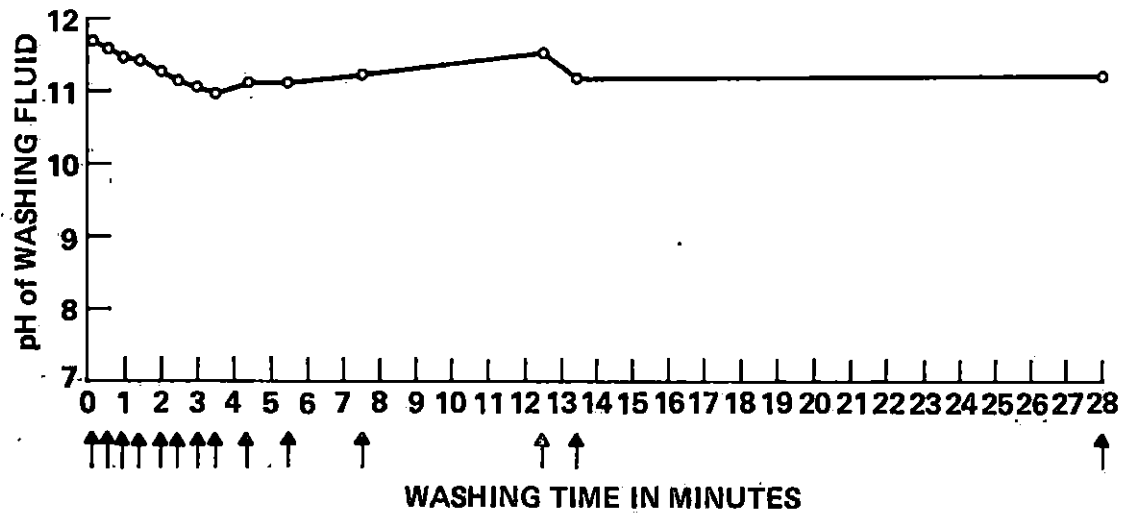
Fig. (4)

Disinfectant Activity Against PRV

The working dilutions of the four disinfectants used through this research work were based on experimental results (Figure 5). A fixed quantity of PRV suspended in PSS titrated against two-fold dilutions of the four disinfectants starting at the RCFU, at 25°C with 5 minutes contact without any fomite was effective through the following dilutions:

- Roccal to 1:128 from RCFU

- Nolvasan to 1:256 from RCFU

- I Stroke Environ to 1:256 from RCFU

- Sodium hydroxide to 1:2048 from RCFU

The 10-fold dilutions required for this study calculated in ppm are presented in Table 9.

Application of the test disinfectants pre- or post-disease appearance, had some positive or negative effects on their disinfecting activity, depending on the disinfectant type (Figure 6). Soiled bedding was chosen to show those effects at 25°C with PSS diluent for 5 minutes contact. Nolvasan and I Stroke Environ were found to be more effective if used before contamination of the fomite with virus. Roccal and sodium hydroxide were more effective when used after the soiled bedding was contaminated with virus.
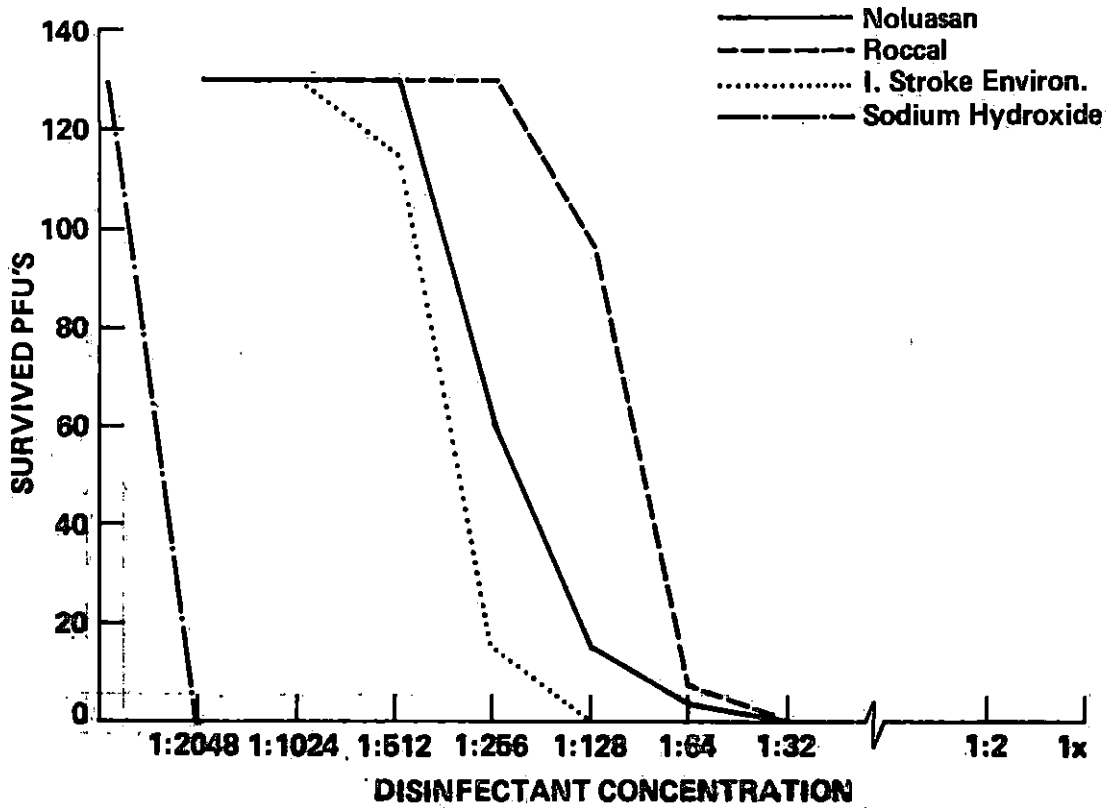
Figure 5.   Survival of PRV in contact with two-fold
dilutions of disinfectants. PSS diluent,
25°C, 5 minutes contact, pipet mixing

Table 9. Dilutions used to study the disinfectant activity against PRV, determined from their manufacturers' recommended concentrations for field use (RCFU)

Dilution used	Disinfectants Concentration (in ppm)			
	Nolvasan[R]	Roccal[R] I	Stroke Environ[R]	Sodium hydroxide
RCFU	23427.5	1600.0	3906.25	21300
I. Two-fold				
1/64	366.2	25.0	61.035	332.8
1/128	183.1	12.5	30.51	166.4
1/256	91.55	6.25	15.25	83.2
1/512	45.775	3.125	7.62	41.6
1/1024	22.88	1.56	3.81	20.8
1/2048	11.44	0.78	1.90	10.4
II. Ten-fold				
1:10	2343.75	160.0	390.62	2130.0
1:100	234.375	16.0	39.06	213.0
1:1000	23.4375	1.6	3.906	21.3

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Figure 6. Effects of pre- and post-disinfection of
PRV-contaminated-soiled bedding on each of
the 4 disinfectants' activity using PSS
diluent and pipet mixing at 25°C for 5
minute virus contact
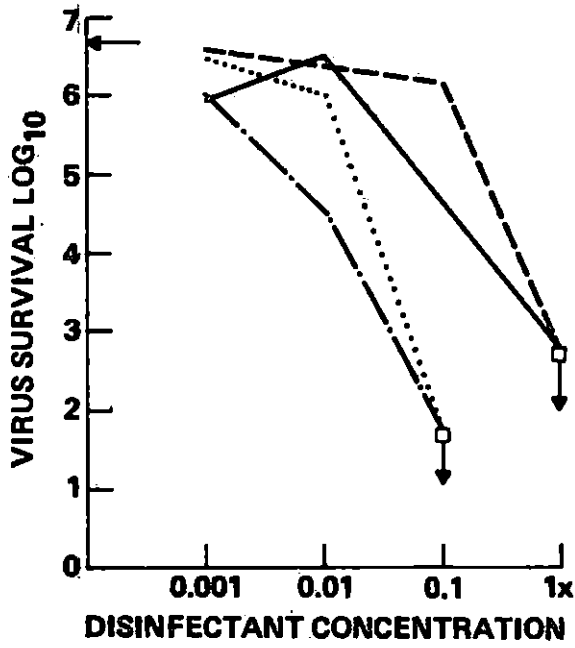
Fig. (6-a)

Nolvasan
Roccal
I. Stroke Environ.
Sod. Hydroxide
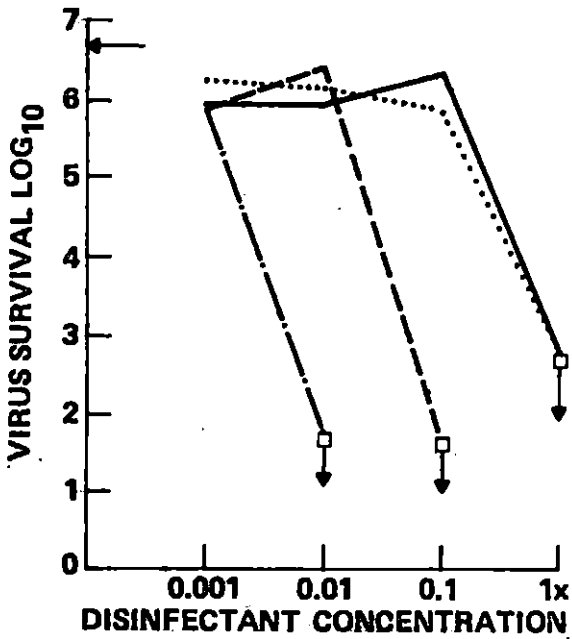
Fig. (6-b)

Effects of Diluents on Disinfectant Activity
Against PRV, with and without Fomites

Pseudorabies virus (PRV) was suspended in 4 diluents
and subjected at 25°C to 4 disinfectants in the absence and
presence of different fomites for 5 or 30 min. contact
time, using pipet mixing (Figures 7-10).

The disinfectant activity against PRV suspended in 4
test diluents at 25°C was tested for 5 min. contact without
any fomite (Figure 7). Nolvasan, Roccal and I Stroke Environ
were effective at 1:10 dilutions of their RCFU against PRV
suspended in PSS or saline G and 1:100 dilutions of their
RCFU against virus suspended in nasal washings. Sodium
hydroxide was effective at 1:10 dilution of its RCFU in
the presence of each of the 4 diluents. Saliva appeared to
be significantly more protective for the virus than the
other test diluents against all 4 disinfectants tested.

Effects of PSS were determined in the presence of
fomites on disinfectant activity (Figure 8). Nolvasan and
Roccal were bound by loam soil and about 90% of their dis-
infecting activity was inhibited by new wood and soiled bedding
as compared with no fomite control. However, in the absence
of these disinfectants, new wood and to a lesser extent,
loam soil were quite toxic to the virus alone in PSS.
About 90% of I Stroke Environ activity, was inhibited
also by the fomites tested. Sodium hydroxide activity

was inhibited by new wood but improved by soiled bedding and rubber with presence of PSS diluent.

Effects of saline G were assessed in the presence of fomites (Figure 9). Nolvasan and Roccal activity were tied up by loam soil and their disinfecting activity reduced about 90% by new wood and soiled bedding in the presence of saline G as diluent. Although the virus titer declined rapidly in contact with new wood without disinfectants (Figure 3), I Stroke Environ activity was improved by new wood but inhibited by loam soil and soiled bedding. Sodium hydroxide retained its activity and no detectable virus at 1:10 dilution from RCFU appeared with or without fomites.

Effects of saliva were determined in the presence of fomites (Figure 10). Nolvasan and Roccal activities were bound by new wood and loam soil, and the virus retained the same titer as seen in the absence of these disinfectants (Figure 3). The activity of Nolvasan and Roccal was approximately 90% inhibited by soiled bedding. Disinfecting activity of Nolvasan and Roccal was however, increased by plastic grate fomite in the presence of saliva as diluent compared to no fomite. I Stroke Environ and sodium hydroxide activities also were bound or inhibited by new wood and improved by plastic grate in the presence of saliva as diluent.

Figure 7.   Effects of each of the 4 disinfectants
            against PRV survival, suspended in
            each of the 4 diluents tested without
            fomite at 25°C for 30 minute contact,
            using pipet mixing

Fig. (7-a) NOLVASAN

Fig. (7-b) ROCCAL

Fig. (7-c) I STROKE ENVIRON

Fig. (7-d) SODIUM HYDROXIDE

PSS

SALINE G.

SALIVA

NASAL WASHING

VIRUS SURVIVAL LOG$_{10}$

DISINFECTANT CONCENTRATION

Figure 8. Effects of PSS diluent on each of the 4
disinfectants' activity against PRV in
contact with each of the fomites tested
at 25°C for 30 minute contact, using
pipet mixing
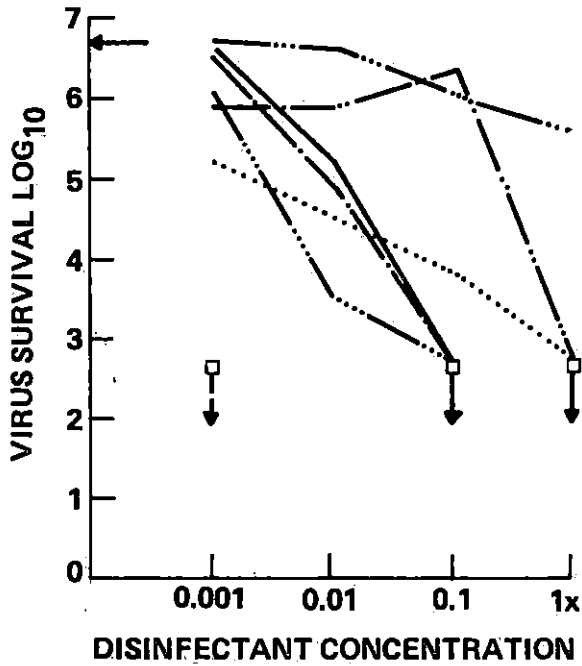
Fig. (8-a) NOLVASAN
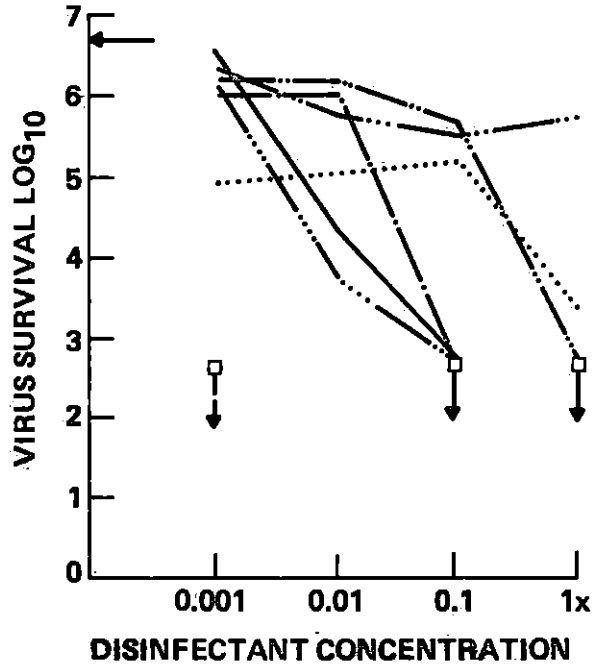
Fig. (8-b) ROCCAL
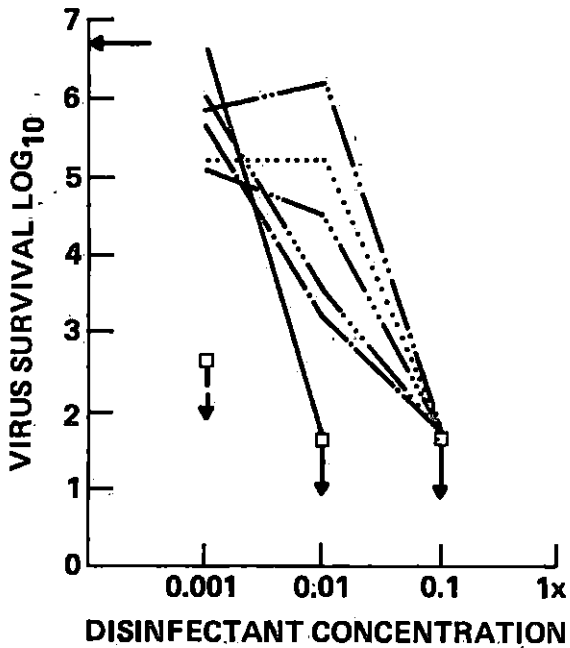
Fig. (8-c) I STROKE ENVIRON.
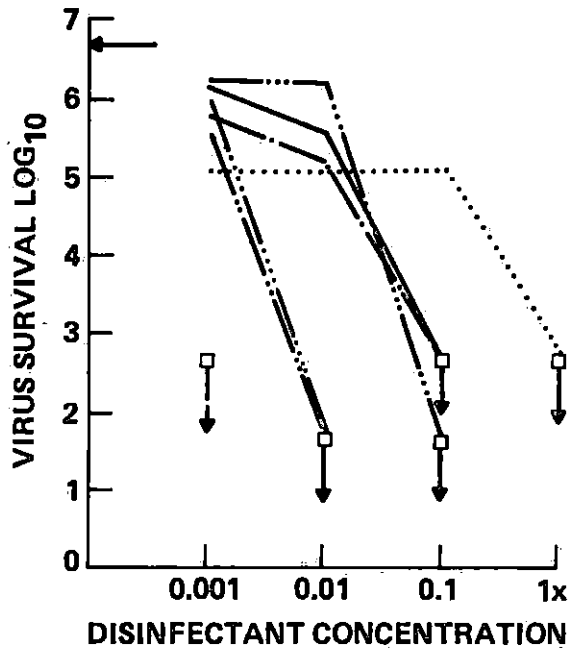
Fig. (8-d) SODIUM HYDROXIDE

Figure 9.   Effects of saline G diluent on each of the
             4 disinfectants' activity against PRV in con-
             tact with each of the fomites tested at 25°C,
             for 30 minute contact, using pipet mixing
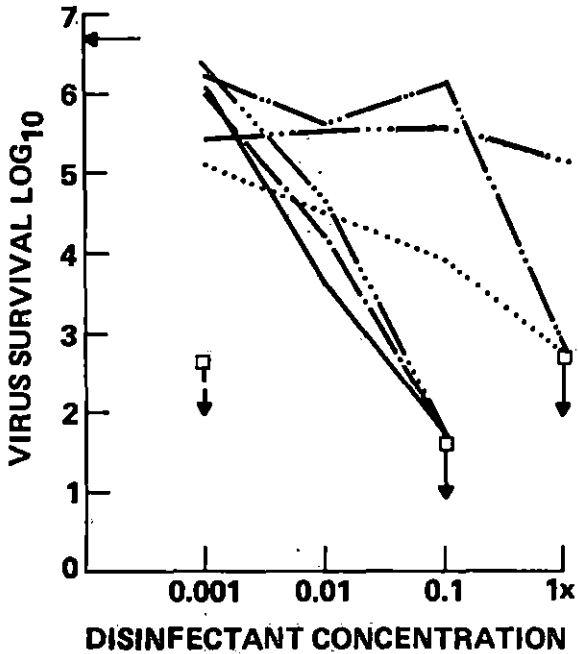
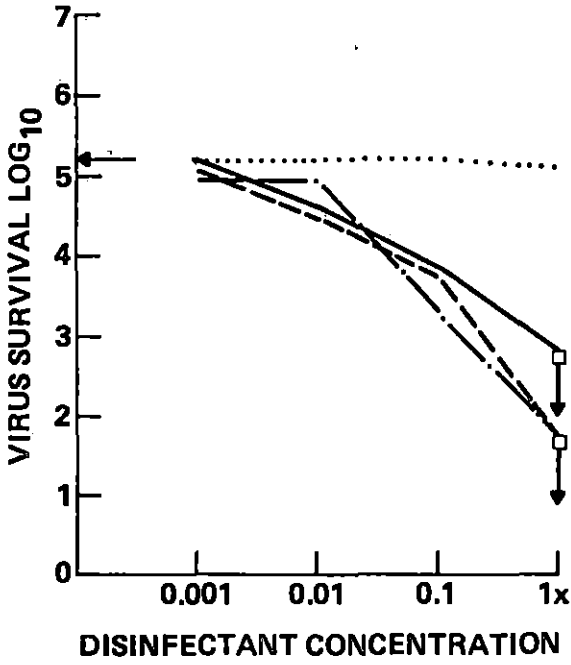Fig. (9-a) NOLVASAN
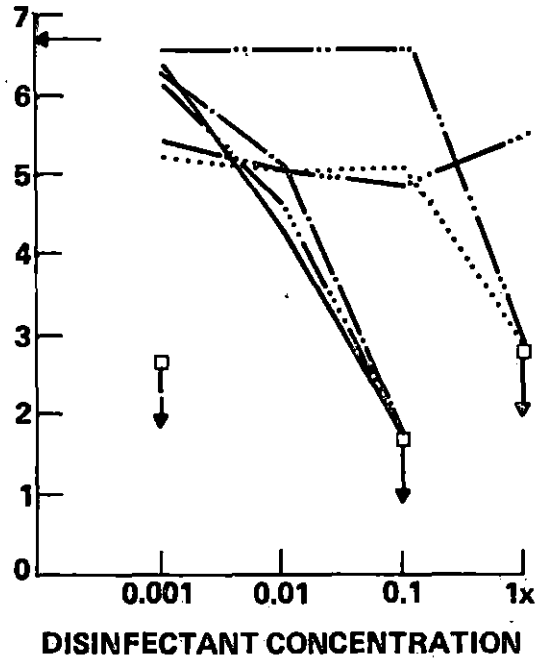
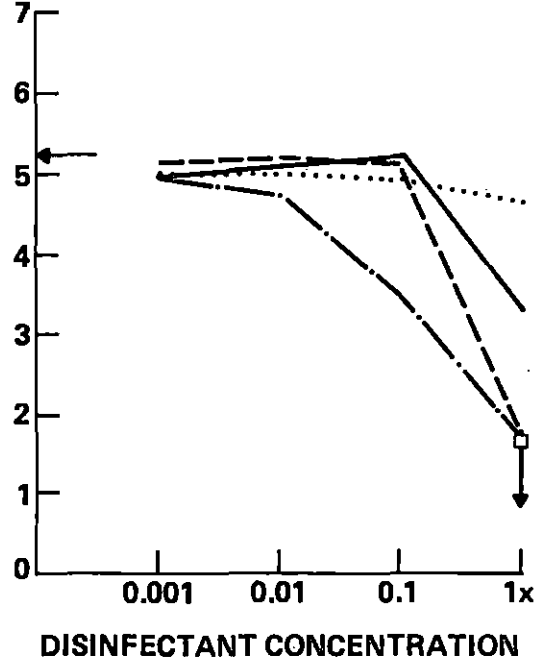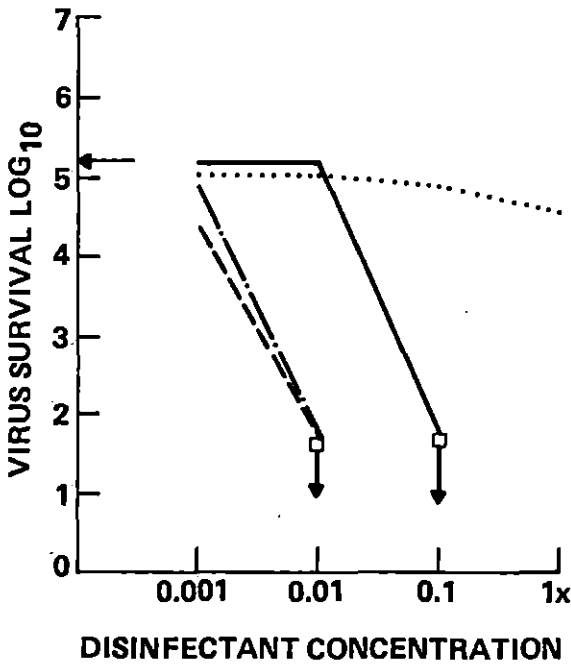Fig. (9-b) ROCCAL
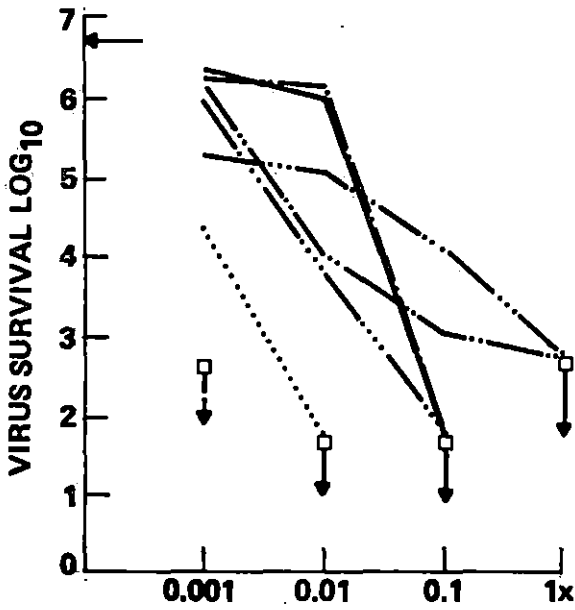
Fig. (9-c) I STROKE ENVIRON.
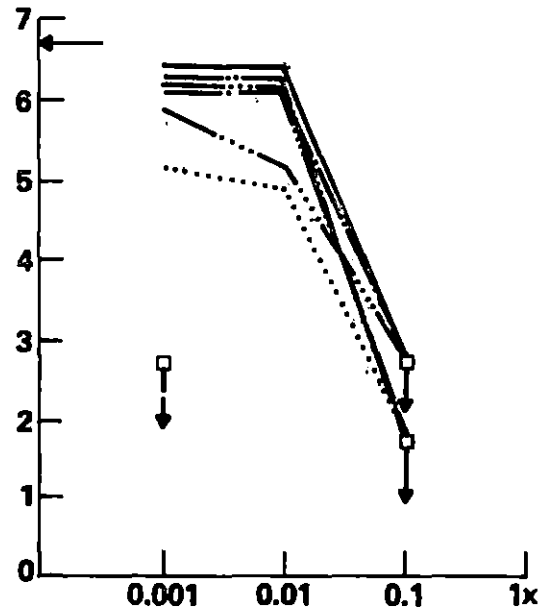
Fig (9-d) SODIUM HYDROXIDE

Figure 10. Effects of saliva diluent on each of the 4
            disinfectants' activity against PRV in con-
            tact with each of the fomites tested at
            25°C for 30 minute contact, using pipet
            mixing
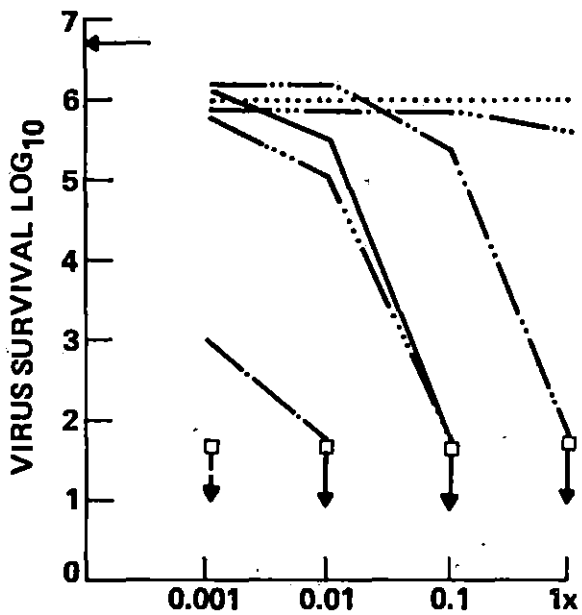
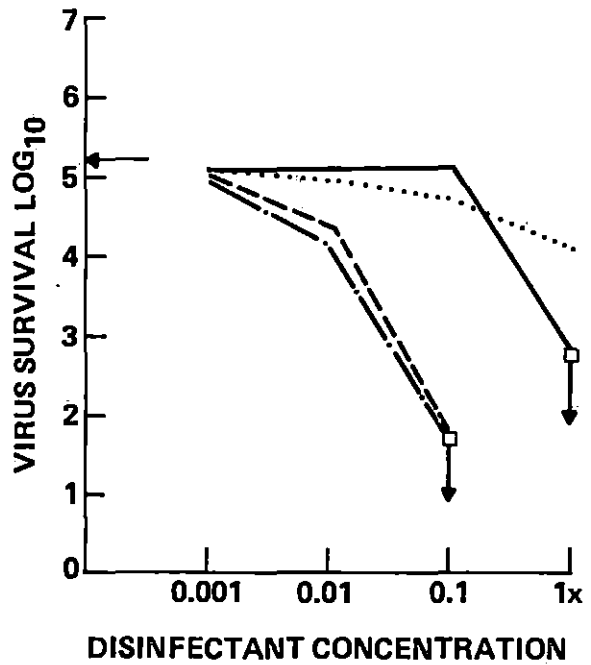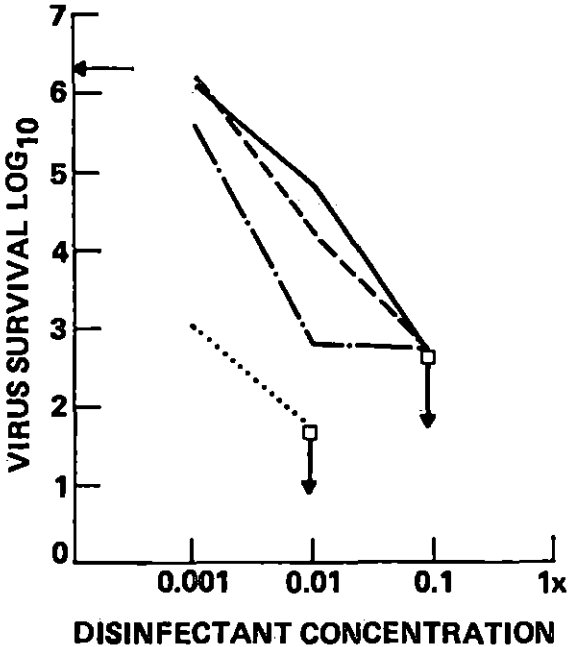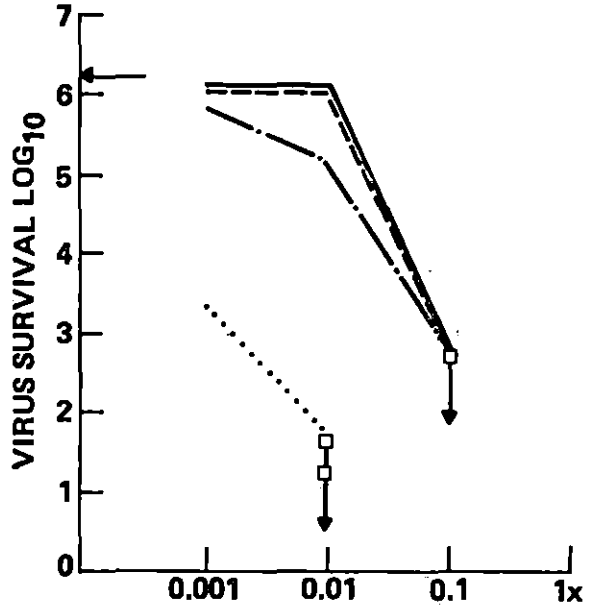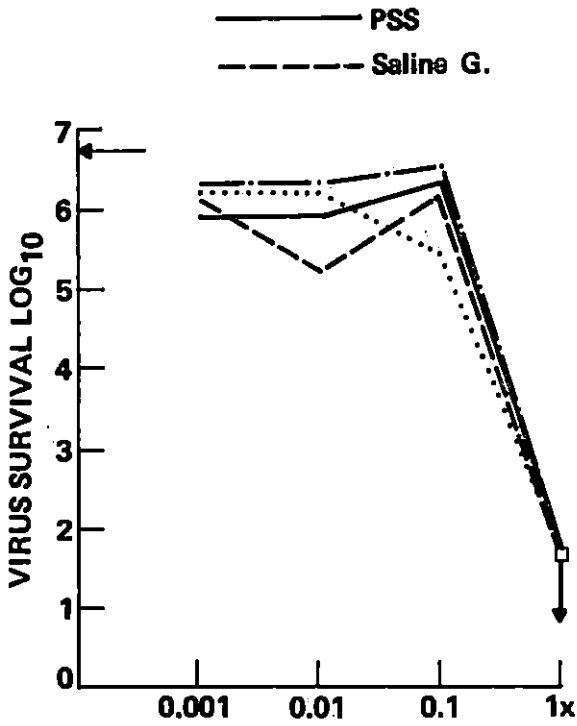Fig. (10-a) NOLVASAN
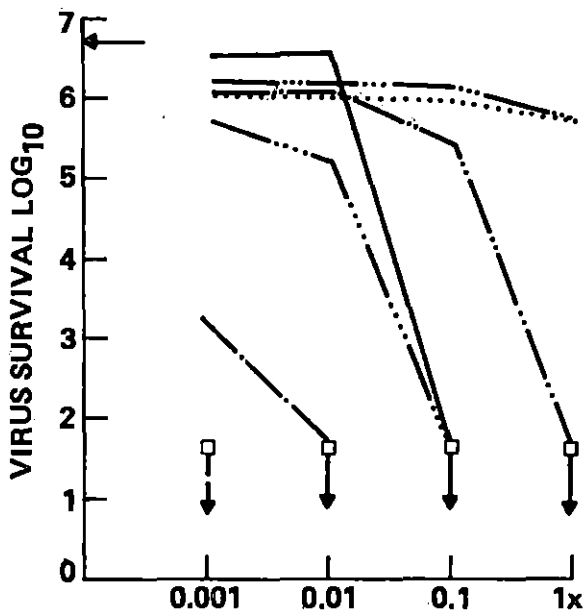
Fig. (10-b) ROCCAL
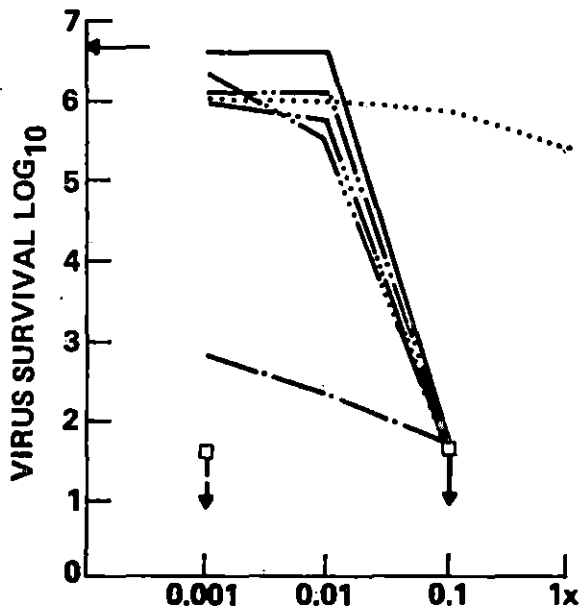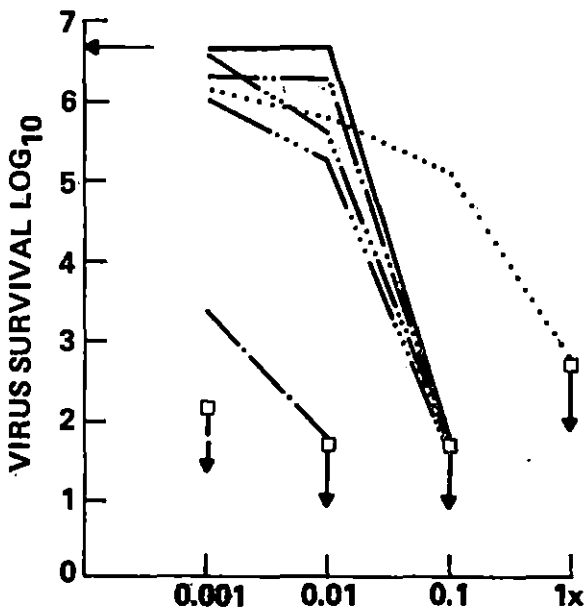
Fig. (10-c) I STROKE ENVIRON.

Fig. (10-d) SODIUM HYDROXIDE

Effect of Fomites on Disinfectant Activity
against PRV in the Four Tested Diluents

The effect of the test fomites on the four disinfectants
against PRV was tested in the presence of four diluents at
25°C for 30 min., using pipet mixing (Figures 11-14).

New wood (Figure 11), in the presence of saliva as
diluent bound all four disinfectants resulting in virus
protection as if there were no disinfectants (Figure 3). In
the presence of PSS, saline G and nasal washings, new wood
resulted in a 10-100-fold decrease in activity of Nolvasan,
Roccal and sodium hydroxide as compared to their action in
the absence of any fomite (Figure 7). However, I Stroke
Environ retained its full activity in the presence of diluents,
other than saliva as if no fomites were present (Figure 7).

Plastic grate (Figure 12) generally had no significant
effect on the action of the four disinfectants in the presence
of PSS, saline G and nasal washing diluents from that of no
fomite (Figure 7). In the presence of saliva, however, plastic
grate significantly improved the activity of the four test
disinfectants (Figure 7). In the absence of disinfectants,
plastic grate in contact with saliva (Figure 3), showed no
difference from saliva alone in viral stability.

Soiled bedding (Figure 13), in the presence of the
four diluents, decreased the activity of Nolvasan, Roccal, and

I Stroke Environ 10-100-fold from their activities with-
out fomite (Figure 7), and no detectable virus was found only
with the use of the disinfectants at their RCFU. However,
the activity of sodium hydroxide was increased 10-fold in
contact with PSS from that without fomite (Figure 7); one-
tenth of the RCFU of this disinfectant completely inactivated
the virus in the presence of all four diluents.

Loam soil (Figure 14), in the presence of the four
diluents, tied up Nolvasan and Roccal and decreased 10-fold
the activity of I Stroke Environ from that without any fomite
(Figure 7). Loam soil,in the absence of disinfectants
(Figure 3), showed virus protection with saliva only, but in
the presence of PSS, saline G and nasal washing, there was
significant inactivation of the virus. Sodium hydroxide
showed no difference in activity against PRV in contact
with loam soil in the presence of the four diluents from
that without any fomite (Figure 7).

<div align="center">

Effect of Temperature with and without
Fomites on Disinfectant Activity
against PRV

</div>

The action of the four test disinfectants against PRV,
with and without fomites, was tested at 4°C and 25°C in the
presence of saline G as diluent for 30 min. contact with
pipet mixing (Figures 15-19).

Figure 11. Effects of new wood fomite on each of the
            4 disinfectants' activity against PRV
            suspended in each of the 4 diluents tested
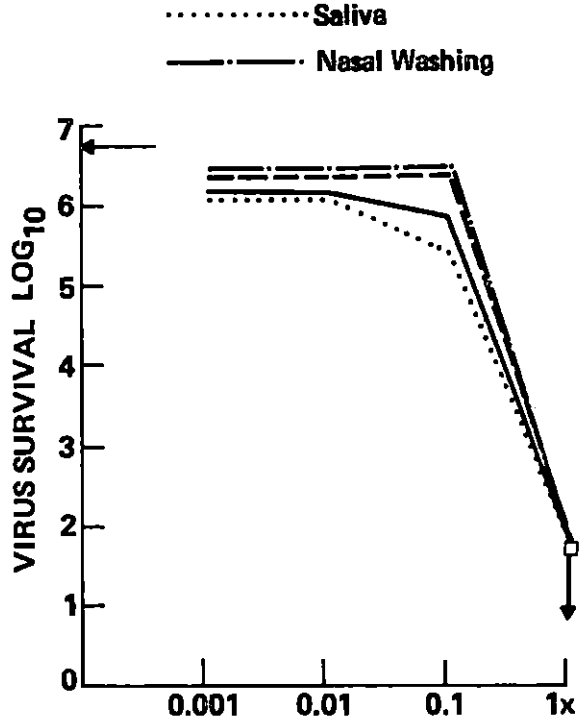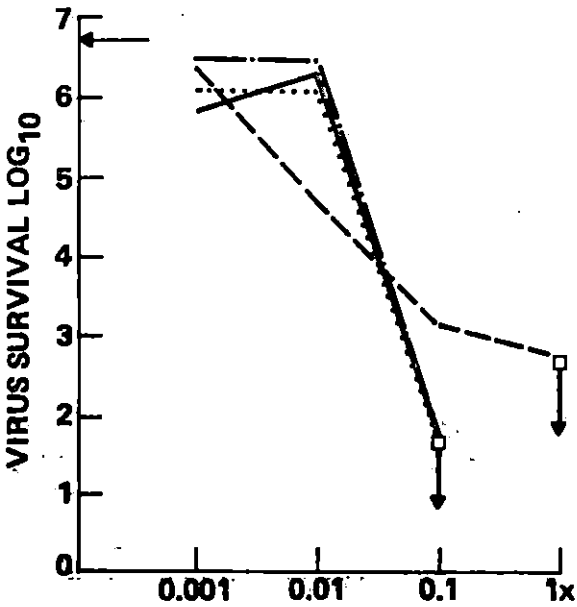   at 25°C for 30 minute contact, using pipet
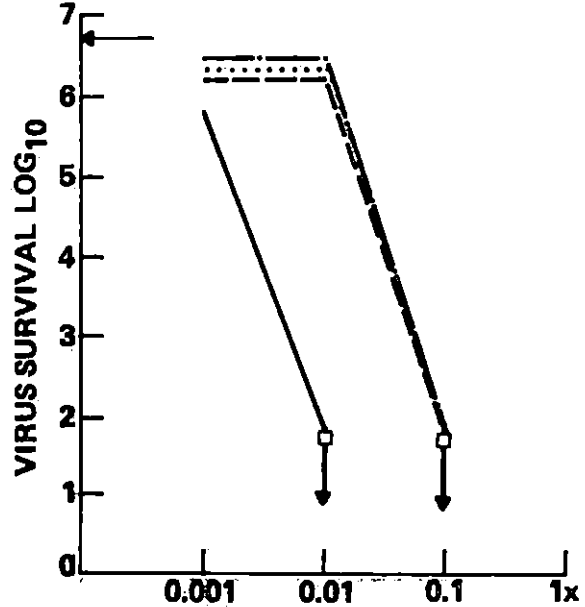   mixing

Fig. (11-a) NOLVASAN
Fig. (11-b) ROCCAL
Fig. (11-c) I STROKE ENVIRON.
Fig. (11-d) SODIUM HYDROXIDE

Figure 12.   Effects of plastic grate fomite on each of
             the 4 disinfectants' activity against PRV
             suspended in each of the 4 diluents tested
             at 25°C for 30 minute contact using pipet
             mixing

Fig. (12-a) NOLVASAN
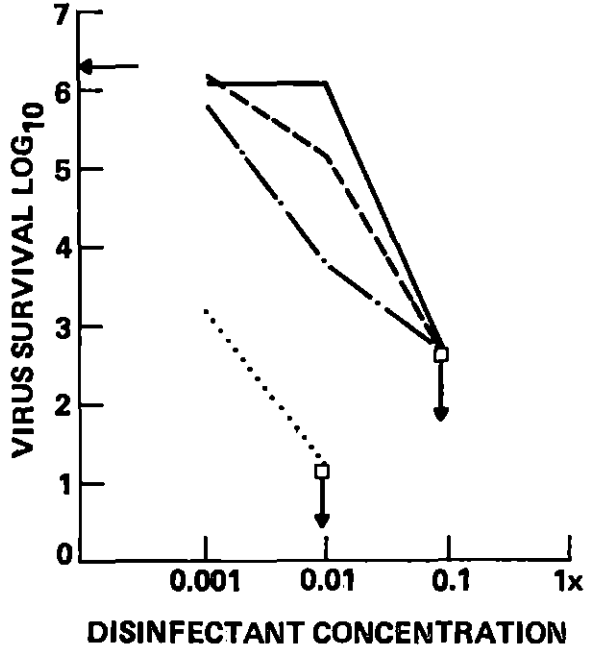
Fig. (12-b) ROCCAL
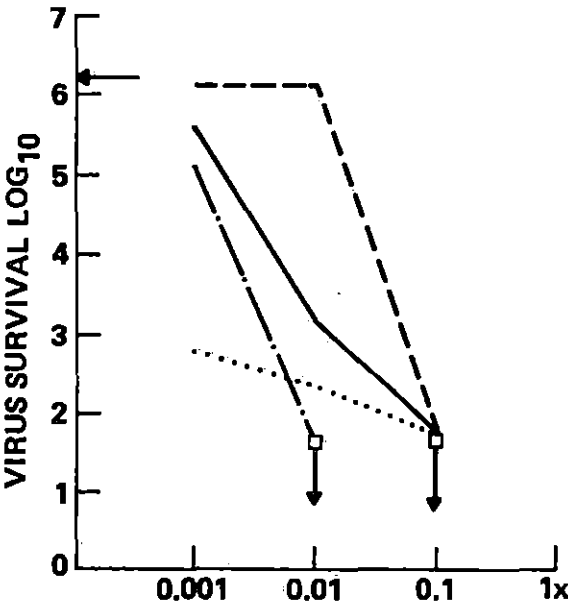
(12-c) I STROKE ENVIRON.

Fig (12-d) SODIUM HYDROXIDE

Figure 13.   Effects of soiled bedding fomite on each of the
             4 disinfectants' activity against PRV sus-
             pended in each of the 4 diluents tested at
             25°C for 30 minute contact using pipet
             mixing

Fig. (13-a) NOLVASAN

DISINFECTANT CONCENTRATION
Fig. (13-b) ROCCAL

DISINFECTANT CONCENTRATION
Fig. (13-c) I STROKE ENVIRON.

DISINFECTANT CONCENTRATION
Fig. (13-d) SODIUM HYDROXIDE

Figure 14. Effects of loam soil fomite on each of the
4 disinfectants' activity against PRV
suspended in each of 4 diluents tested
at 25°C for 30 minute contact using pipet
mixing

Fig (14-a)
DISINFECTANT CONCENTRATION

(14-b)
DISINFECTANT CONCENTRATION

DISINFECTANT CONCENTRATION
Fig. (14-c) I STROKE ENVIRON.
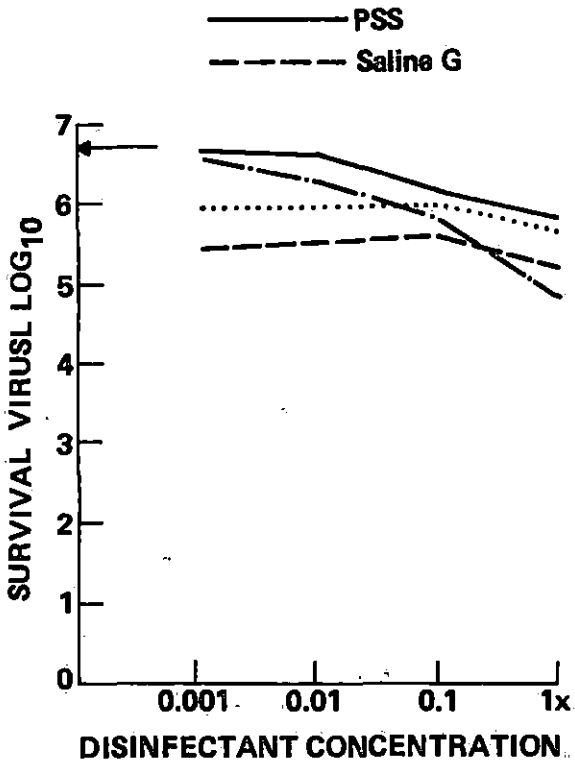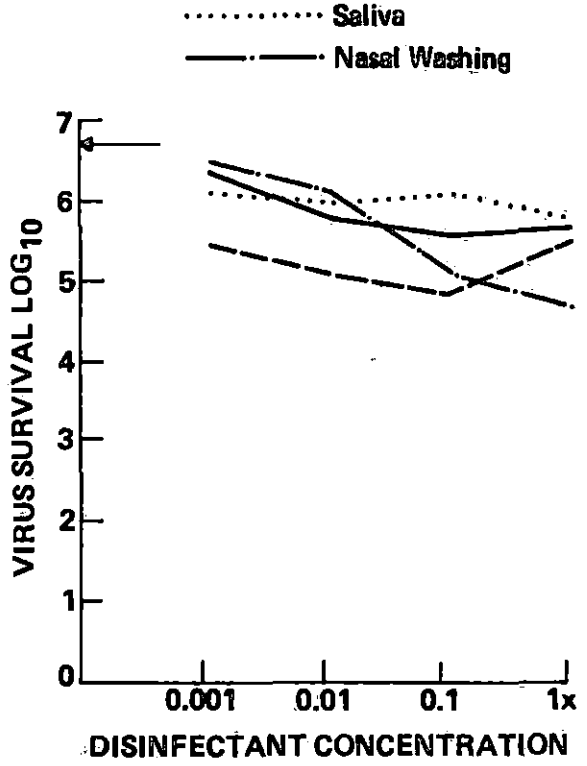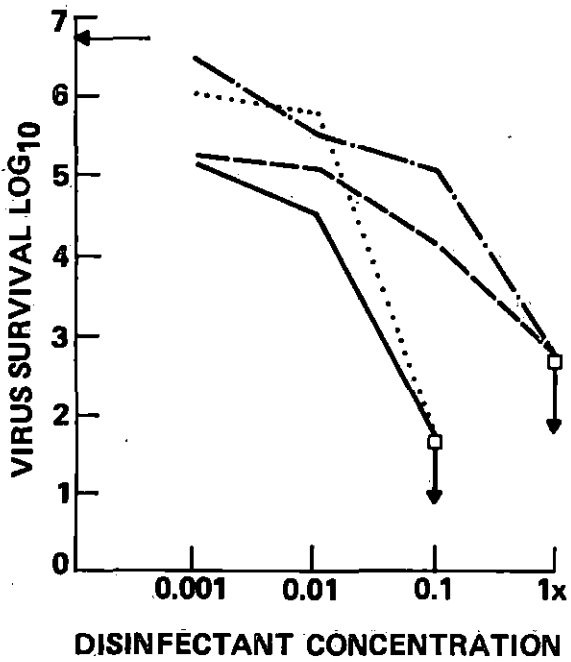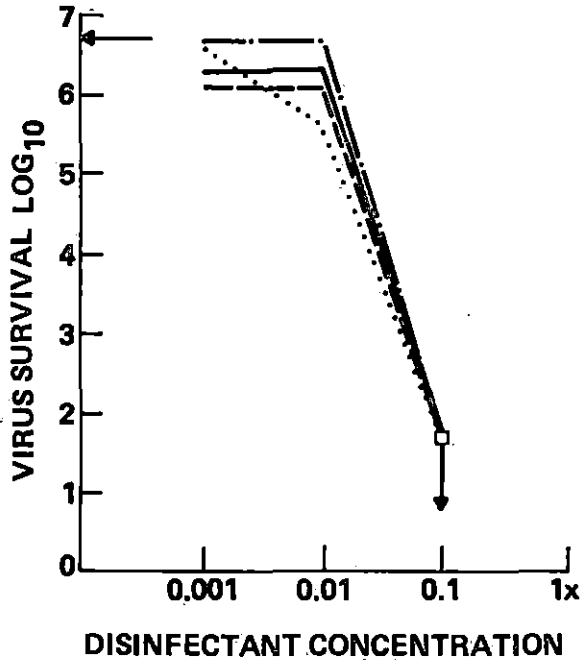
DISINFECTANT CONCENTRATION
Fig (14-d) SODIUM HYDROXIDE

Without any fomite (Figure 15), Nolvasan was more effective at 25°C, but there was no significant temperature effect on Roccal, I Stroke Environ or sodium hydroxide at 4° or 25°C. No detectable virus was recovered at either temperature from the four disinfectants at 1:10 dilution of their RCFU.

With new wood (Figure 16), Nolvasan and Roccal showed about 90% lowering of their disinfectant activity from that without any fomite but no differences were recorded at either temperature and no detectable virus remained at RCFU. I Stroke Environ was 10-fold more active at 25°C, with no detectable virus at 1:100 dilution (39 ppm) while 1:10 dilution (390.6 ppm) was needed to render the virus undetectable at 4°C. Sodium hydroxide was slightly more effective in the absence of any fomite (Figure 15) at 1:100 dilution, but no detectable virus was recovered at either temperature at 1:10 dilution of its RCFU.

With plastic grate (Figure 17), Nolvasan and Roccal showed better activity at 25°C. I Stroke Environ and sodium hydroxide showed no difference at either temperature. The four disinfectants were as effective in the presence as in the absence of any fomite (Figure 15) and no detectable virus was found at 1:10 dilution of their RCFUs.

With soiled bedding (Figure 18), Nolvasan showed slightly

better activity in the higher dilutions at 25°C, but in lower
dilutions, no significant difference was recorded between
its activity at the two temperatures. Roccal showed no
significant difference in activity at either temperature
and no detectable virus remained at the RCFU. I Stroke
Environ also showed rapid inactivation of PRV in higher
dilution at 25°C. At 4°C, no detectable virus remained at
1:10 dilution (390.6 ppm), while at RCFU (3906.5 ppm) at
25°C, the virus was undetectable. Sodium hydroxide showed
no significant difference in activity at either temperature
and no detectable virus remained at 1:10 RCFU at either
temperature.

With loam soil (Figure 19), Nolvasan and Roccal showed
more activity in higher dilutions at 4°C, and no significant
difference at lower disinfectant dilutions between the two
temperatures. I Stroke Environ was 10-fold more effective
at 4°C than at 25°C, with no detectable virus at the RCFU
at 25°C or at 1:10 dilution at 4°C. Sodium hydroxide was
more effective in higher dilutions at 4°C than at 25°C
but in lower disinfectant dilutions, no detectable virus
appeared at either temperature.

Figure 15.   Effects of temperature on each of the 4
             disinfectants' activity against PRV sus-
             pended in saline G diluent without fomite
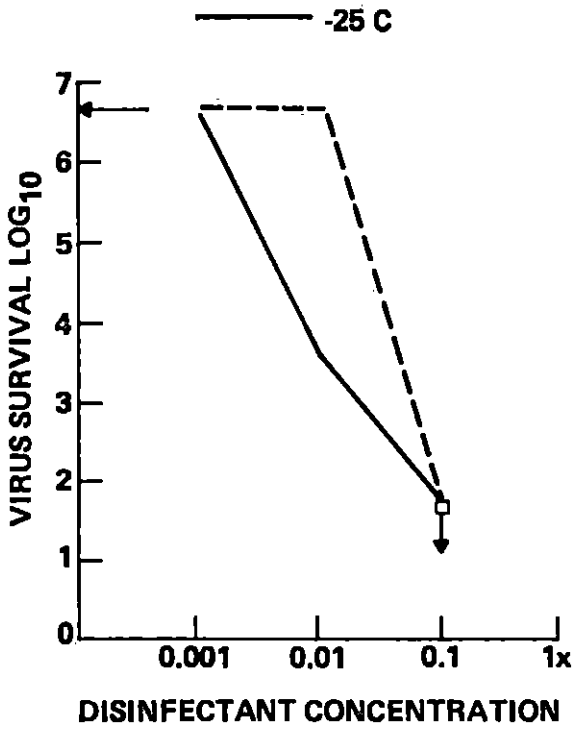   at 4° and 25°C for 5 minute contact using
             pipet mixing
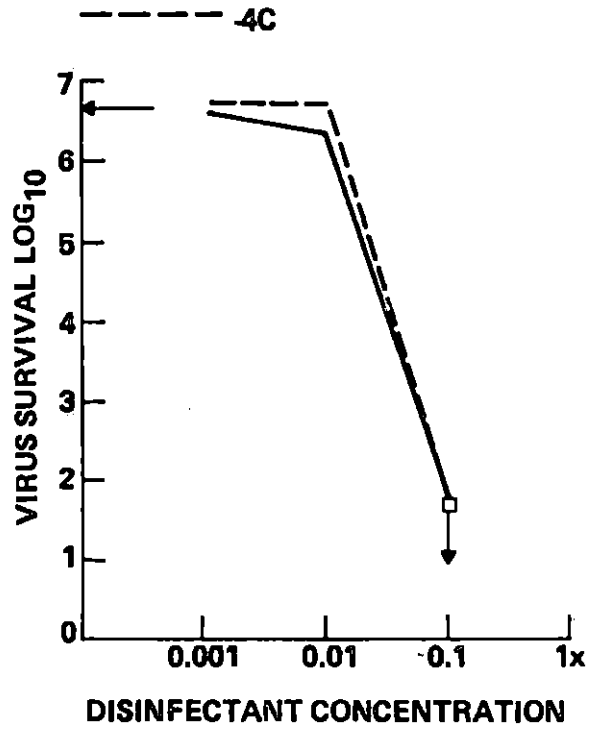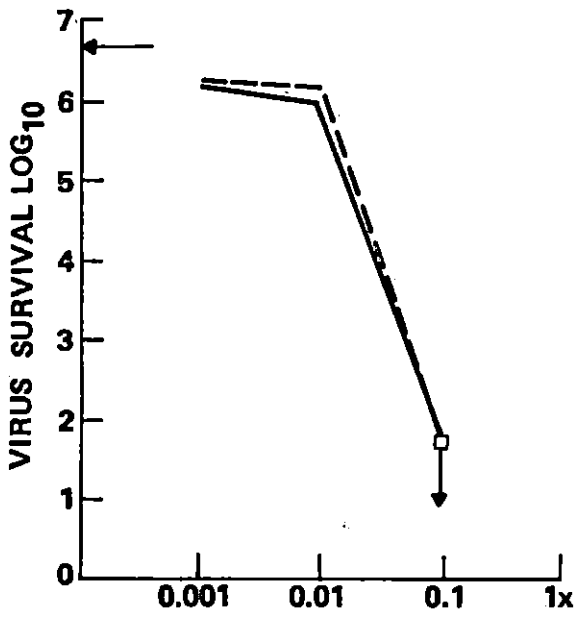
Fig. (15-a) NOLVASAN
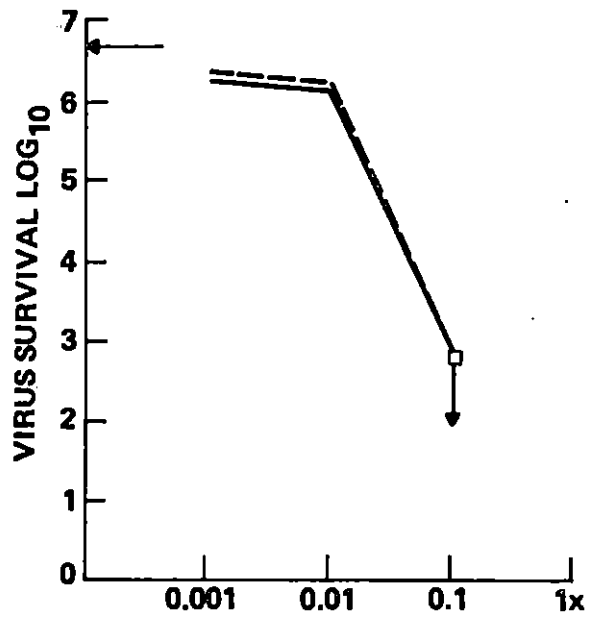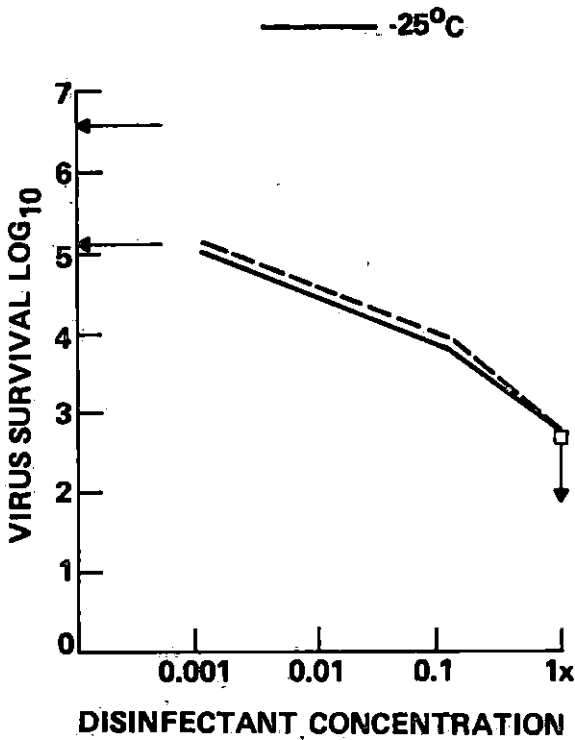
Fig. (15-b) ROCCAL

Fig. (15-c) I STROKE ENVIRON.

Fig. (15-d) SODIUM HYDROXIDE

Figure 16. Effects of temperature on each of the 4
disinfectants' activity against PRV sus-
pended in saline in contact with new
wood at 4° and 25°C for 30 minutes
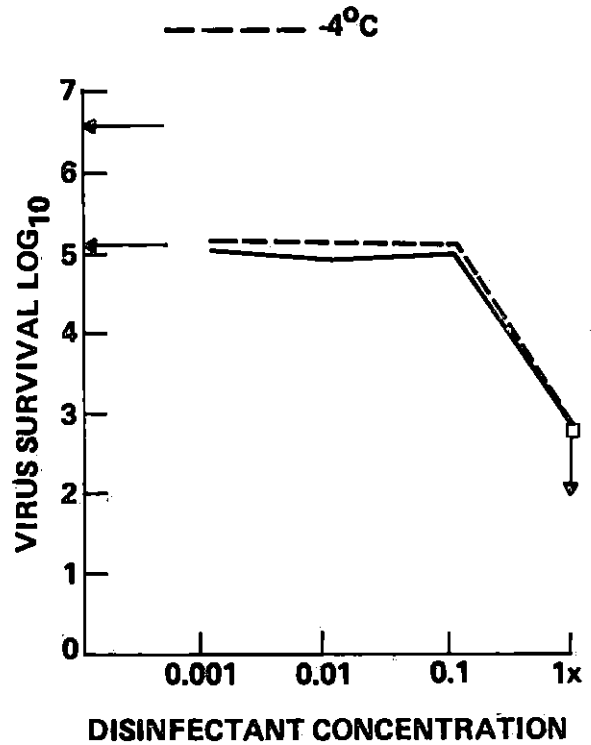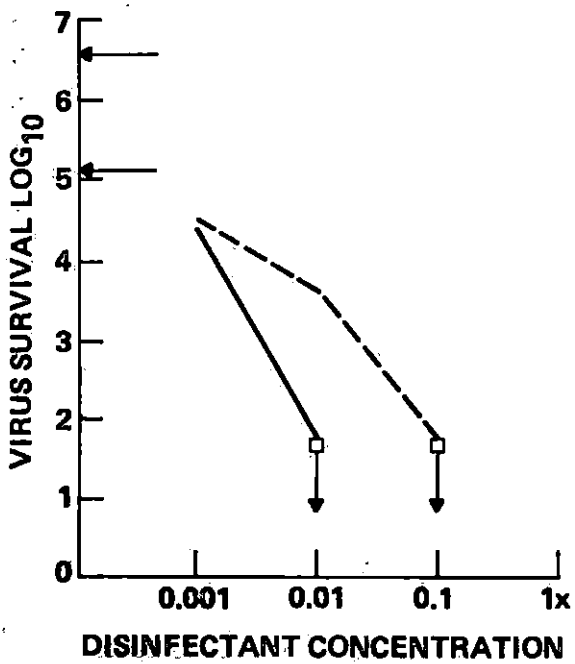using pipet mixing

— 25°C   — — — -4°C

Fig. (16-a) NOLVASAN

Fig. (16-b) ROCCAL

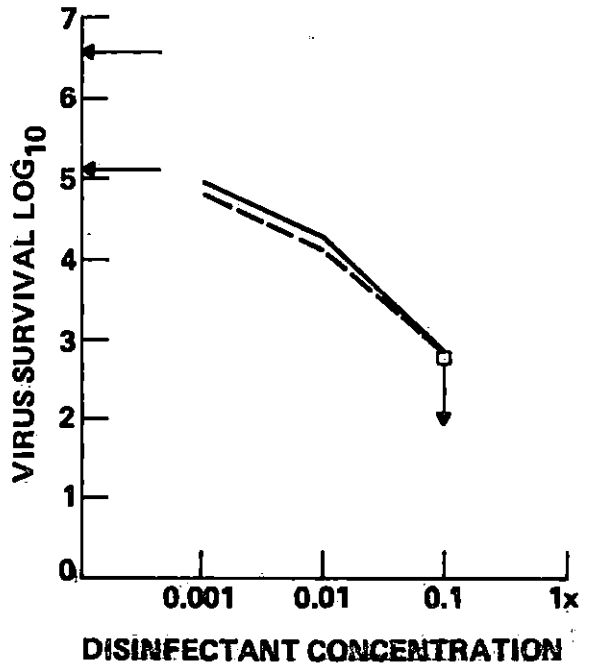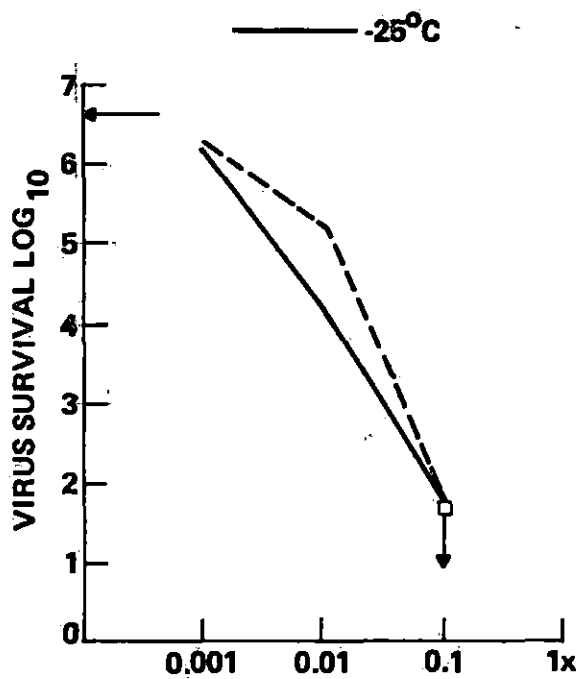Fig. (16-c) I STROKE ENVIRON.
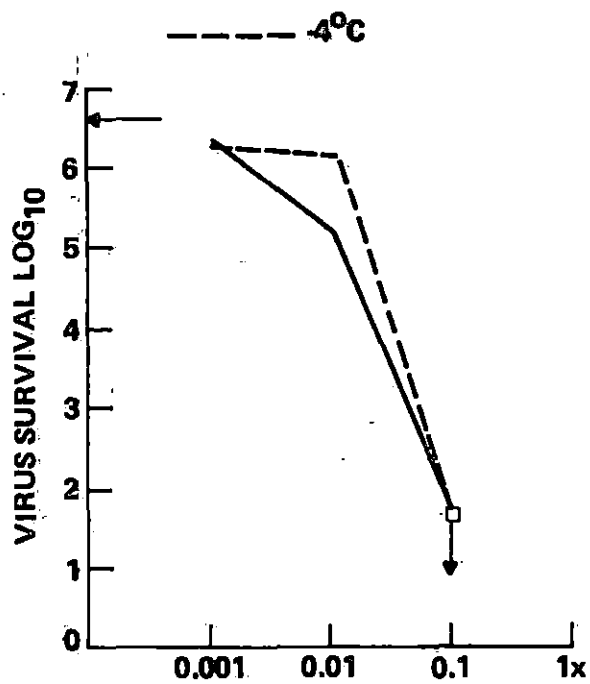
Fig. (16-d) SODIUM HYDROXIDE

Figure 17.   Effects of temperature on each of the 4
              disinfectants' activity against PRV suspended
              in saline G and in contact with plastic grate
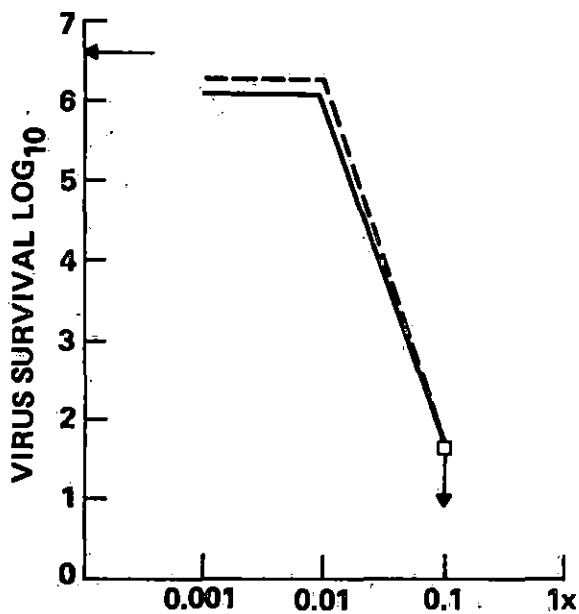   at 4° and 25°C for 30 minutes using pipet
              mixing

Fig. (17-a) NOLVASAN

Fig. (17-b) ROCCAL
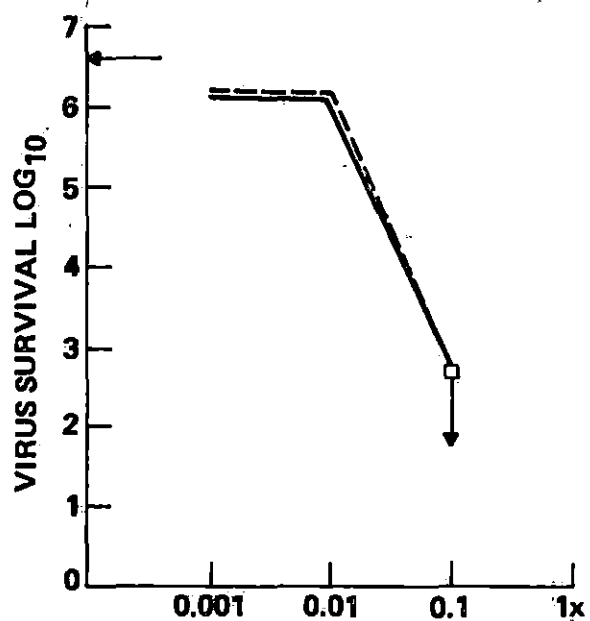
Fig. (17-c) I STROKE ENVIRON.

Fig. (17-d) SODIUM HYDROXIDE

Figure 18.   Effects of temperature on each of the 4
             disinfectants' activity on PRV suspended in
             saline G and in contact with soiled bedding
             at 4° and 25°C for 30 minutes using pipet
             mixing

Fig (18-a) NOLVASAN

Fig. (18-b) ROCCAL

Fig (18-c) I STROKE ENVIRON.

Fig. (18-d) SODIUM HYDROXIDE

Figure 19. Effects of temperature on each of the 4
            disinfectants' activity against PRV suspended
            in saline G and in contact with loam soil at
            4° and 25°C for 30 minutes using pipet
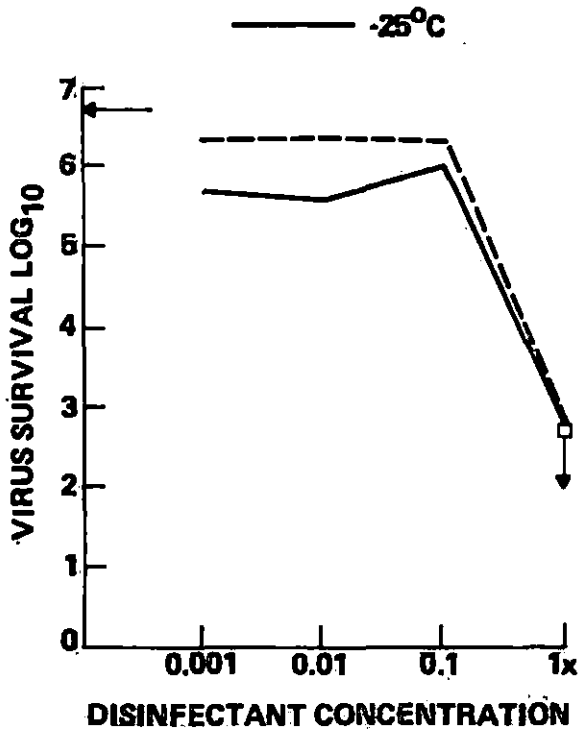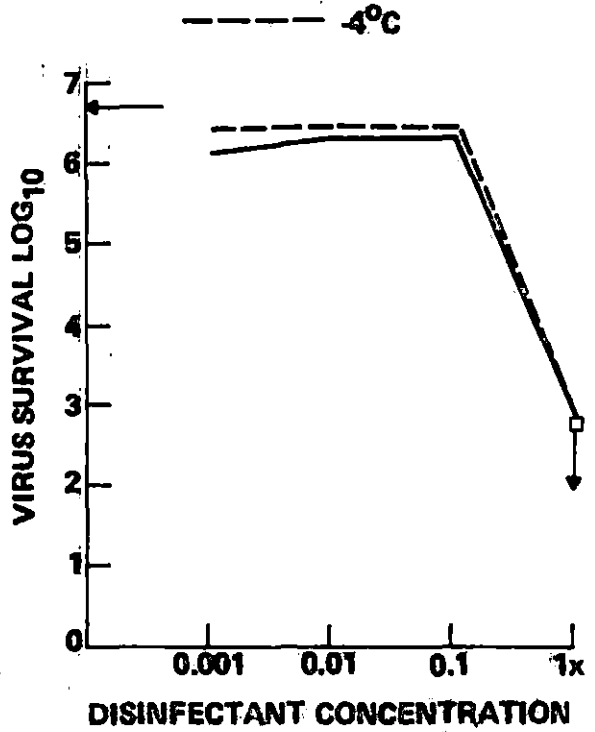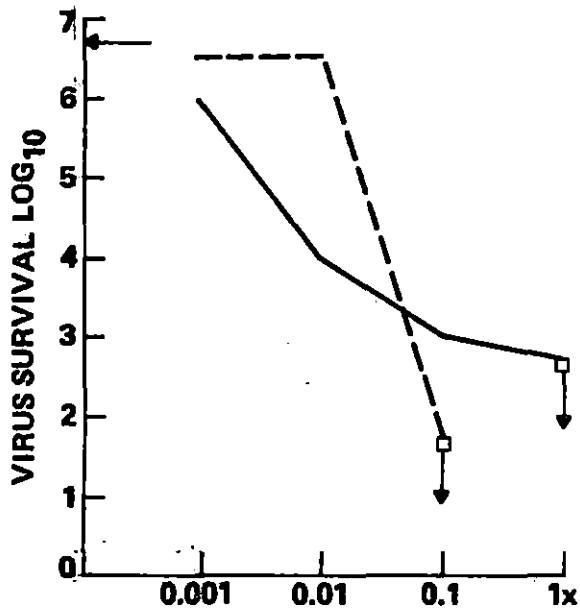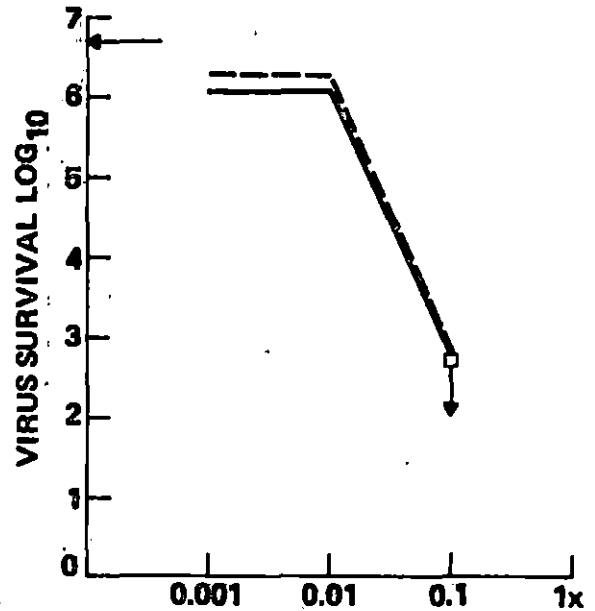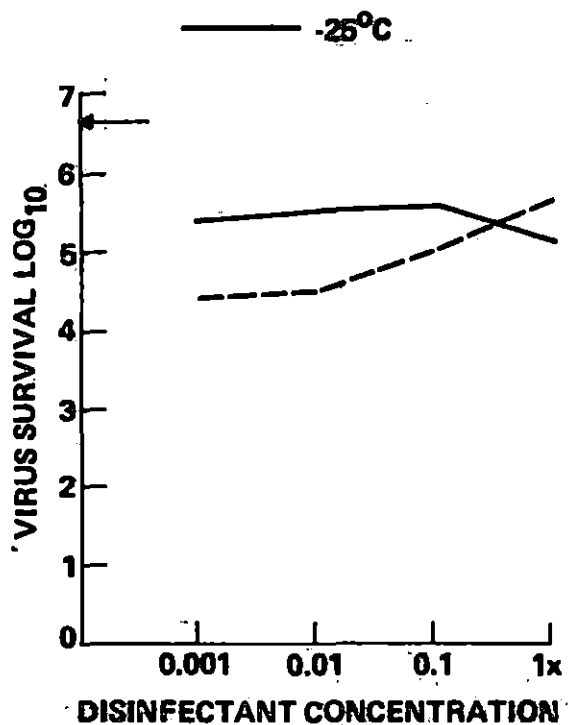   mixing
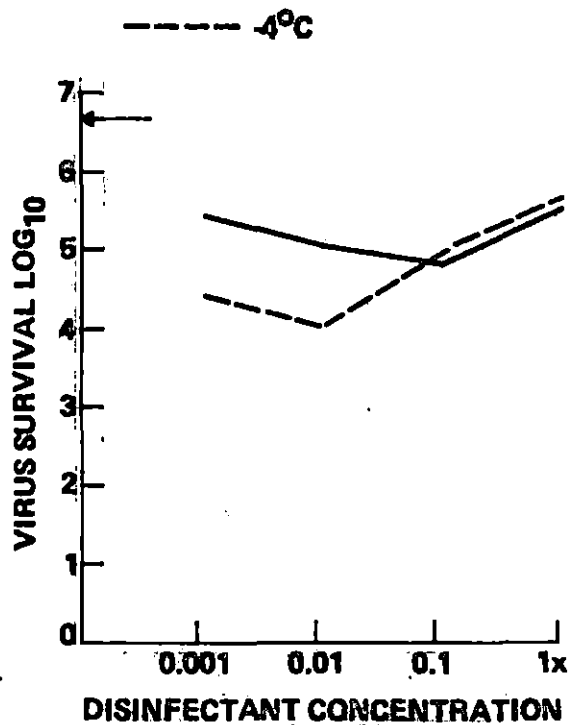
— -25°C   ---- -4°C

Fig. (19-a) NOLVASAN

Fig. (19-b) ROCCAL
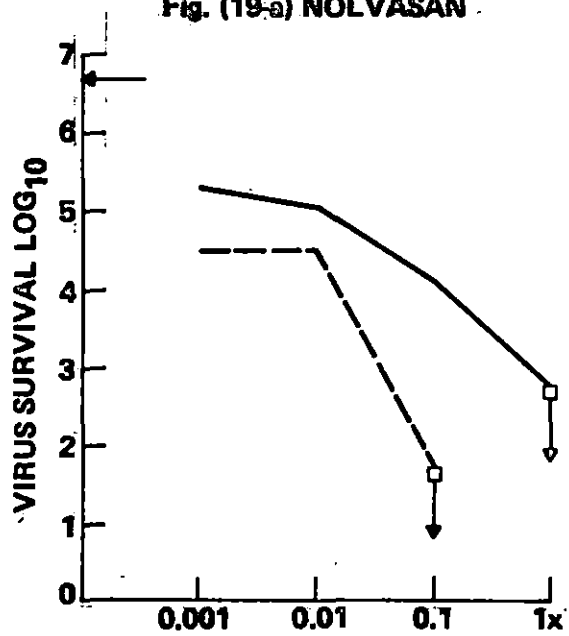
Fig. (19-c) I STROKE ENVIRON.
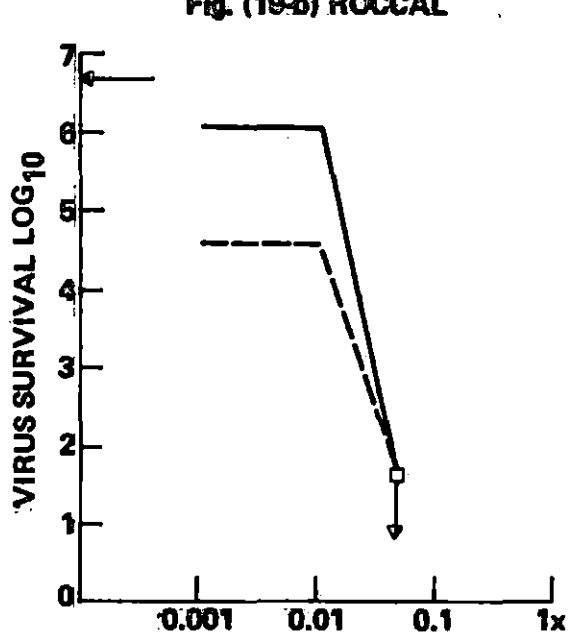
(19-d) SODIUM HYDROXIDE

DISCUSSION

Pseudorabies virus (PRV) survival outside the living
host as well as some environmental factors that might affect
its survival, have been discussed in previous studies (32,
44). The direct activities of many types of disinfectants
against the virus have been well-documented and are dis-
cussed in the literature review. It has also been well-
documented that environmental transmission of PRV to
pigs occurs, and that pigs have been infected after being
housed in animal barns which previously housed pigs with
PRV infections. This research work was designed to study
the activity of disinfectants in the presence of environmental
factors, and to assess the interactions of disinfectants and
environmental fomites in inhibiting the viral inactivating
actions of both the fomites and the disinfectants, permitting
PRV to survive longer at infectious levels.

The disinfectants used in this study represent four
main categories of chemical compounds that have been mostly
used for farm disinfection, namely chlorhexidine, quaternary
ammonium compounds, phenol derivatives and alkalies. Those
disinfectants have been found to be effective against PRV
by many research workers as discussed in the literature review
part of this writing.

As an enveloped virus in which the envelope is assembled

from the nuclear membrane, PRV is sensitive to a wide range
of disinfectants and can be inactivated easily by disin-
fectants or agents with lipid dissolving capability, like
Nolvasan and I Stroke Environ, cationic compounds like
Roccal, alkalies like sodium hydroxide, as well as by
anionic detergents and acids. Disinfectants, other than
lipid solvents, also may act by disrupting the structure
and ionization of the amine and carboxyl radicals of the
virus envelope, resulting in destruction of virus attach-
ment sites, and thus, inhibiting viral infectivity. However,
in actual use, the activities of disinfectants may be in-
hibited by environmental components which might be found in
the surrounding vicinity or where the virus is present, such
as serum, blood, tissues, body fluids or other fomites that
might inhibit disinfectants' actions.

Four diluents were used in this study. PSS and saline
G, in which the virus was most stable at neutral pH, and at
temperature near 25°C, while porcine saliva and nasal washing
showed toxic inhibitory effects against the virus. These
findings have been previously reported (44).

In the absence of fomites, the four disinfectants used
in this study, Nolvasan, Roccal, I Stroke Environ and sodium
hydroxide were found to be active against PRV; $>10^6$ $TCID_{50}$
of the virus suspended in each of the four diluents were

fully inactivated within 30 min. at 25°C by 1:10 to 1:100 dilutions of the RCFU of those disinfectants in all of the test diluents. However, porcine saliva and saline G, which either by their protein content, or buffer activity, respectively, protected the virus from low disinfectant concentrations; but porcine nasal washings with their toxicity added to the disinfectant activity, resulting in rapid virus inactivation at low disinfectant concentration. Only sodium hydroxide showed similar activity irrespective of the suspending fluids, inactivating the virus at 1:10 dilution of its recommended concentration in all test diluents.

PRV suspensions at pH levels mentioned (34) as favorable for PRV survival remained stable in the presence of plastic grate, loam soil and soiled bedding; concrete raised the pH; and new wood lowered the pH to levels at which virus was inactivated. Among these fomites, concrete and plastic grate enhanced the action of disinfectants tested; the other fomites inhibited disinfecting activity.

Clean concrete in the presence of PSS, saline G and nasal washing diluents generated a pH (11.8 ± 0.4) that resulted in rapid virus decay; disinfectant activity was greatly enhanced, instantly inactivating the virus. Saliva, which showed some inhibiting or deteriorating activity on PRV by itself, somewhat protected the virus from

concrete activity, but the concrete-disinfectant combined activity overcame the protection of saliva, leading to inactivation of the virus instantly also.

The PRV inactivating action of concrete was retained even with continuous washing with PSS and hydrochloric acid neutralization. But 10X concentration of PBS, a concentrated buffer solution with pH 7.0, controlled concrete activity and brought the pH down to 8.0 $\pm$ 0.2, where the virus survived without significant difference from the no fomite control within the time of the experiment. However, vigorous shaking generated additional concrete activity (pH 9.1) that resulted in >2 logs loss from the virus titer. Thus, the alkaline pH of concrete was considered to be the main contributing factor to virus decay, both by itself and in the presence of the test disinfectants as well. By and large, clean concrete retained its activity and capability of PRV inhibition for a long period of time that enhanced disinfectant activity.

Plastic grate fomite was previously shown (44) to inhibit PRV, especially in the presence of saliva. In the presence of plastic grate, disinfecting activity was enhanced, especially in the presence of saliva diluent, where the virus inactivation was uniquely rapid. The regular inhibiting activity of saliva, which was increased in the

presence of plastic grate, greatly increased the activity
of all four disinfectants against PRV. The exact mechanism
was not clearly understood; it was considered that there
might be increased saliva enzymatic activity in the presence
of plastic that resulted in increased virus vulnerability
to disinfectant activity. Some increase in disinfectant
activity against PRV was also shown by plastic grate fomite
in the presence of nasal washing, saline G and PSS. Thus,
plastic grate could be disinfected readily with any of the
four test disinfectants without any problems.

Soiled bedding, due to the presence of organic material,
decreased the activity of Nolvasan and Roccal against PRV
by more than 90% in the presence of all four diluents; with
only the test concentrations at their RCFU being effective
in inactivating the virus. Thus, the use of these two
disinfectants to disinfect soiled bedding would require con-
centrations at least at their RCFU levels; otherwise, they
would not be effective. A 1:10 dilution of I Stroke Environ
disinfectant resulted in no detectable virus in the presence
of PSS, nasal washings and saliva diluents with soiled bedding,
but, in the presence of saline G, this disinfectant lost
about 90% of its activity and had to be applied at its RCFU
for disinfecting soiled bedding. Sodium hydroxide retained
its activity in contact with soiled bedding in the presence

of all four test diluents. Thus, sodium hydroxide and I Stroke Environ were effective for disinfecting soiled bedding if maintained in a good disinfecting concentration levels.

New wood, in the presence of PSS, saline G and nasal washings, decreased the activity of Nolvasan and Roccal against PRV by more than 90%, an effect also noted with sodium hydroxide in the presence of PSS. Only at their RCFUs were these disinfectants able to inactivate the virus. However, no significant change in I Stroke Environ activity against PRV was recorded by new wood in the presence of PSS, saline G or nasal washing diluents. In the presence of saliva, new wood bound the activities of all four disinfectants, maintaining the virus at an infectious level. Even at the RCFU strength of these disinfectants, the virus titer (initially >6.76 logs) was decreased only 1, 0.5, 0.4 and 0.25 logs, by the activities of sodium hydroxide, I Stroke Environ, Roccal and Nolvasan, respectively. This protective action of new wood was not present in the absence of disinfectants where new wood caused a rapid decline in the virus titer by itself in the presence of PSS, saline G and nasal washings, and a small decline by itself in the presence of saliva. This showed that wooden walls, bars, doors or bedding from wood shavings which have viral inactivating action in themselves might actually protect the

virus in the presence of saliva from the disinfectant activity of all four test compounds.

Loam soil tied up Nolvasan and Roccal at all dilutions tested, even at RCFU, protecting the virus from disinfectant action, especially in saliva and PSS, although the soil was in itself toxic to the virus. In loam soil, I Stroke Environ inhibited the virus at 1:10 dilution of its RCFU in the presence of PSS and saliva, but only at RCFU did it inhibit the virus in the presence of nasal washing and saline G. Sodium hydroxide retained its activity and inhibited the virus at 1:10 dilution of its RCFU in all diluents. Thus, Nolvasan and Roccal were not effective for disinfecting loam soil; I Stroke Environ might be helpful if maintained in high concentration and not diluted in soil to below the effective concentration. However, sodium hydroxide stood as the most effective disinfectant for loam soil against PRV.

The effects of temperature on disinfectant activity were tested at 4° and 25°C in the presence of saline G with and without fomites. Without fomites, Nolvasan showed more activity at 25°C than at 4°C. Roccal, I Stroke Environ and sodium hydroxide showed no difference in their activity at either temperature. With new wood, only I Stroke Environ was more effective at 25°C than at 4°C, other disinfectants showed no difference in their activities against PRV between

the two temperatures tested. With plastic grate, Nolvasan
and Roccal were more active at 25°C, I Stroke Environ and
sodium hydroxide showed no difference. Soiled bedding showed
I Stroke Environ to be more effective at 25°C, and no dif-
ference in the activity of the other disinfectants at either
temperature. With loam soil, all four disinfectants were
more effective at 4°C, especially I Stroke Environ. Thus,
fomites other than loam soil were preferably disinfected
at 25°C with the disinfecatnt of choice for that fomite.
Loam soil was most effectively disinfected at 4°C, with
I Stroke Environ and sodium hydroxide being the most active
of the test disinfectants on it.

CONCLUSIONS

Findings in this study have application in disease
control of importance for the livestock industry. This work
showed that the mere use of chemical disinfectants in farm
environments does not mean that infective agents were
destroyed or transmission of disease controlled. Depending
on the environmental conditions, specific disinfectants
may effectively inactivate infectious agents, may have
little or no effectivity, or may even help in the protec-
tion of such agents in the environment. Clean concrete was
effective in inactivating pseudorabies virus by itself, and
with the use of low concentrations of any of the four dis-
infectants tested here, quickly inactivated PRV to below
the detectable level. Thus, concrete should be kept clean
and this will maintain its activity for a long time, acting
together with the test disinfectants. Plastic grate as well
showed no problem of disinfection, and could be easily dis-
infected with any of the test disinfectants. Soiled bedding
reduced the antiviral activity of Nolvasan and Roccal and
to a lesser extent of I Stroke Environ and sodium hydroxide.
High levels of disinfectant were required to inactivate PRV
in the presence of soiled bedding. New wood if kept clean,
was capable of inactivating PRV in the absence of any pro-
tecting fluid by itself. Saliva, however, which is the

main natural carrier for PRV from shedding swine, was pro-
tective to the virus in contact with new wood and also pro-
tected it in a high infectious level from the action of
the four disinfectants tested. Although I Stroke Environ
was not inhibited by new wood in the presence of PSS,
saline G and porcine nasal washing, the other test dis-
infectants lost more than 90% of their activities when
absorbed by the wood fomite. New wood, or wood in general
should be scrubbed very clean to promote its natural inhibi-
tion of PRV and then disinfected with chemical disinfectants
in high concentrations, as they quickly lose their effectivity
on wood below their RCFU. I Stroke Environ and sodium
hydroxide were the most effective against PRV in saliva on
new wood among the test disinfectants, especially at 25°C.
With loam soil, Nolvasan and Roccal failed to show any sig-
nificant effect against PRV in the presence of any of the
four test diluents at either test temperature and thus, could
not be recommended for this job. I Stroke Environ and sodium
hydroxide were effective, if maintained at their RCFU, as
soil disinfectants against PRV, especially at 4°C where they
were more effective than at 25°C.

Generally, surfaces contaminated with PRV which are to
be disinfected should be scrubbed, cleaned, washed and dried,
before chemical disinfection to maintain the activity of

these disinfectants at their RCFU. Any of the four disin-
fectants was effective in disinfecting clean concrete
and plastic. I Stroke Environ and sodium hydroxide were
preferable for new wood and soiled bedding and were the
only disinfectants showing effectivity for loam soil dis-
infection.

Though a swine raising industry is not of economic
importance in Iraq, the herpes viruses may become important
in the very large poultry, cattle, sheep and goat industries.
Much of the information gained in this study may have direct
application to the control of diseases caused by herpes
and other lipid containing viruses as well as those caused by
other infectious agents in Iraq. The techniques developed
for and used in this study may have application for local
studies in my country.

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