Effect of Temperature, Relative Humidity and Medium on the Aerosol Stability of Infectious Bovine Rhinotracheitis Virus

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ABSTRACT

Aerosols of infectious bovine rhinotracheitis virus were generated with a Devilbiss 40 nebulizer from Eagle's minimum essential medium, nasal secretion from a noninfected calf and nasal secretion from a calf artificially infected with infectious bovine rhinotracheitis virus and aged in a rotating drum at temperatures of 6°C or 32°C and relative humidities of 30% or 90%. The aerosols were sampled at seven minutes after start of spraying, one hour, two hours and three hours with an all glass impinger (AGI-30) and titrated for infectivity in cell cultures. Physical decay was determined by a rhodamine B tracer technique. During spraying (seven minutes from start of spraying), the virus was usually more stable in aerosols of nasal secretion from a noninfected calf and at 90% relative humidity. In nasal secretion from a noninfected calf the virus survived best at 90% relative humidity when the temperature was 6°C and best at 30% relative humidity when the temperature was 32°C. During aging, biological decay was greater at the higher temperature, and at 6°C, the highest decay rates occurred at 30% relative humidity in Eagle's minimum essential medium and at 90% relative humidity in nasal secretion from a noninfected calf. The stability of infectious bovine rhinotracheitis virus in infected nasal secretion was not widely different from that in noninfected nasal secretion, although under certain conditions greater survival occurred in the noninfected secretion.

RÉSUMÉ

Cette expérience visait à produire des aérosols contenant le virus de la rhino-trachéite infectieuse bovine, avec un vaporisateur Devilbiss 40, à partir du milieu essentiel minimum d'Eagle, des sécrétions nasales d'un veau témoin et de celles d'un veau expérimentalement infecté avec ce virus. Elle visait également à laisser vieillir ces aérosols, dans un cylindre rotatif, à une température de 6° ou 32°C, combinée à une humidité relative de 30% ou 90%. On préleva des échantillons de ces aérosols avec un appareil en verre (AGI-30), aux intervalles suivants: sept minutes, une, deux et trois heures après la vaporisation; on les ensemença ensuite dans des cultures cellulaires. On en détermina la dénaturation physique, à l'aide d'une technique de marquage à la rhodamine B. Au bout de sept minutes de vaporisation, le virus s'avéra ordinairement plus stable dans les aérosols des sécrétions nasales du veau témoin, à une humidité relative de 90%; il y survécut le mieux à une humidité relative de 90%, jointe à une température de 6°C, ou à une humidité relative de 30%, jointe à une température de 32°C. Au cours du vieillissement, la décomposition biologique se révéla la plus grande à la température la plus élevée; à la température de 6°C, le taux le plus élevé de décomposition se produisit à une humidité relative de 30%, pour ce qui est du

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milieu essentiel minimum d'Eagle, et à une humidité relative de 90%, dans le cas des sécrétions nasales du veau sain. La stabilité du virus de la rhino-trachéite infectieuse bovine dans les sécrétions nasales du veau expérimental ne différa pas beaucoup de sa stabilité dans celles du veau témoin; certaines conditions favorisèrent cependant une meilleure survie du virus dans les sécrétions nasales du veau témoin.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is recognized as a common respiratory pathogen of cattle. Transmission by the airborne route has been demonstrated in feedlot holding cattle clinically ill with respiratory disease (O'Connell and Darcel, unpublished data, 1974). Information about the stability of this virus in air is of significance to epidemiological studies of respiratory disease in cattle. This information is incomplete. Songer (11) studied the effect of relative humidity (RH) on the viability of aerosols of IBR virus generated from Eagle's minimum essential medium (EMEM) and stored at a constant temperature (23°C) while Donaldson and Ferris (6) determined th effect of a range of RH values on the viability of IBR virus in aerosols held at room temperature for periods of one second and five minutes. In the present study, aerosols were generated from EMEM and stored at temperatures of 6°C and 32°C and at RH of 30% and 90%. In addition, the viability of aerosols generated from nasal secretion from a non-infected calf and from a calf infected with IBR virus was determined under the above conditions of temperature and RH, in order to obtain data which might be more relevent to the airborne spread of IBR under field conditions. The aerobiological procedures used were based on those described by Songer (11).

MATERIALS AND METHODS

Virus Cultivation and Assay

The Cooper strain of IBR virus (13) at a low cell culture passage level was used. A stock of the virus produced in embryonic bovine lung (EBL) cell cultures had an infectivity titre of 10^7 median tissue culture infectious doses (TCID 50) per ml and was concentrated to 10^9 $TCID_{50}$/ml by ultrafiltration using an Amicon PM 30 filter under a pressure of 20 lb/inch^2 (14). Viral assays were performed on complete monolayer cultures of second or third passage EBL cells cultivated in minimum essential medium with Hank's salts supplemented with 10% foetal calf serum (FCS-Gibco) in Linbro microtiter plates. The maintenance medium was EMEM with 5% FCS, buffered with HEPES at a concentration of 0.045 M. Tenfold dilutions of samples to be titrated for infectivity were prepared in maintenance medium and 0.1 ml of each dilution was inoculated into each of eight wells in the microtiter plate. The infectivity titre of the sample was then calculated by the Spearman-Kaerber procedure.

Collection of Bovine Nasal Secretion

Bovine nasal secretion (NIBS), from a colostrum-deprived calf which was maintained in strict isolation and lacked antibody against IBR virus, was collected (10) and stored at –70°C. For the production of infected bovine nasal secretion (IBS), a colostrum-deprived 18 months old calf whose serum and nasal secretion were free from IBR virus neutralizing antibodies was inoculated with 2 ml of stock IBR virus in each nostril. Nasal secretion free from antibody against IBR virus was collected on the first and second days on which clinical signs of IBR were apparent.

Aerosol Procedure

The aerosols were held in a 200 litre stainless steel rotating (3 r.p.m.) drum, in which the temperature and RH were continuously monitored (11). For experiments at low RH (30% ± 3) the drum was filled with dry air from a compressed air tank (Canox Ltd). High RH (90% ± 2) was obtained in the drum by atomizing distilled water. The drum was vented during filling and housed in a chamber in which the temperature was held at 6°C or 32°C in different experiments. Virus stock was diluted 10^{-2} in EMEM, NIBS or IBS. A Devilbiss 40 nebulizer with a capacity of

0.3 ml/min and operated at a pressure of 6.5 psi was used to generate aerosols from the above suspending media. The generation time was five minutes, followed by a stabilization period of two minutes. Aerosol samples were taken at seven minutes after start of generation and then at hourly intervals for three hours postgeneration. Aerosol samples were collected for 18 sec with an AGI-30 (16) containing 20 ml of 1% peptone as collecting medium. At each RH and temperature, three or five replicates, respectively, were carried out for virus assay or physical tracer studies.

Determination of Physical Decay

In order to differentiate between biological and physical decay of IBR virus aerosols, rhodamine B was used as a tracer. In a preliminary study using an Andersen sampler and the method of Couch *et al* (2) it was observed that when IBR or rhodamine B was generated from EMEM the virus and rhodamine B distribution in the aerosol according to particle size was not significantly different. The same was true when they were generated from NIBS or IBS. Rhodamine B at a concentration of 2.5 mg/ml in NIBS, IBS or EMEM was aerosolized into the drum. The generation and stabilization time as well as the other aerobiological procedures and protocol used were identical to those described above. Rhodamine B concentrations in the nebulized media and in the impinger fluids or from the Andersen sampler were determined by fluorometric analysis (11). The physical aerosol spray loss was defined as rhodamine B concentration per litre of cloud sprayed minus the concentration of the tracer in the impinger fluid at seven minutes. The physical aerosol storage loss was the difference between the concentration of tracer in the impinger fluid at seven minutes and in the impinger fluids collected at one, two and three hours.

Determination of Particle Size of Aerosols

Since particle size plays an important role in aerosol stability and determines the site of deposition of inhaled aerosols in the respiratory tract, the size of the aerosol particles produced by the nebulizer in the drum was measured to ensure the production of aerosols of appropriate particle size. Rhodamine B at a concentration of 2.5 mg/ml in NIBS or EMEM was aerosolized into the drum as described above, at 30% and 90% RH and temperatures of 6°C and 32°C. The aerosols were sampled seven minutes after generation as described above, except that two parallel AGI-30 were used and a pre-impinger (9) was mounted on the inlet of one AGI-30 in order to retain particles $> 5\ \mu$ and to restrict the sample collected in this AGI-30 to particles $\leqslant 5\ \mu$. The second AGI-30 was operated without a pre-impinger in order to sample the aerosol without size discrimination. Fluorometric analysis of the fluids collected in each AGI-30 was carried out and the percentage of aerosol particles with a diameter $\leqslant 5\ \mu$ (PAP $\leqslant 5\ \mu$) was calculated according to the formula:

$$\text{PAP} \leqslant 5\,\mu = \frac{\text{Rhodamine B recovery in AGI-30 with pre-impinger}}{\text{Rhodamine B recovery in AGI-30 without pre-impinger}} \times 100$$

The effect of media on the PAP $\leqslant 5\ \mu$ was analysed by one-way analysis of variance and Duncan's multiple range test (12), at the 5% level of significance.

Calculation and Statistical Analysis of Biological Decay Rates

The decrease in virus concentration due to loss of viral infectivity in the air (biological decay) was expressed as biological decay rate. The biological decay rate during the spray period (0.7 min) was computed according to the formula $\log \frac{No - \log Nt}{t}$, in which log No represents the virus concentration at time 0 calculated from the volume of suspension which was nebulized, log Nt is the concentration of virus recovered at seven minutes postgeneration and t is time in hours (0.116 hr). The biological decay rate (regression coefficient) during the storage period (seven minutes to three hours) was calculated by the method of least squares (12). Virus decay rates in aerosols of EMEM, NIBS and IBS during spray and storage were subjected to the analysis of variance (12) to compare the effect on virus decay rate of medium at each temperature and humidity combination, of temperature within the same medium and RH and of RH within

TABLE I. Size of Aerosol Particles Generated from Bovine Nasal Secretion of Noninfected Calf (NIBS) and Eagle's Minimum Essential Medium (EMEM) at Seven Minutes Postgeneration at Different Temperatures (T) and Relative Humidities (RH)

	Percentage Aerosol Particles with a Diameter $\leq 5\mu$ ($\pm$SD)[a]			
	NIBS		EMEM	
T	30% RH	90% RH	30% RH	90% RH
32°C	92.7 ± 1.0[b]	97.6 ± 0.9[c]	97.5 ± 0.9[b]	94.4 ± 3.6[c]
6°C	97.7 ± 0.8	88.7 ± 1.4[b]	97.3 ± 0.7	94.4 ± 1.8

[a]SD = standard deviation
[b]Particle size from EMEM is significantly >BNS
[c]Particle size from BNS is significantly > EMEM

TABLE II. Recovery of Infectious Bovine Rhinotracheitis Virus from Aerosols of Nasal Secretion from Noninfected Calf (NIBS) Eagle's Minimum Essential Medium (EMEM) at Different Temperatures (T) and Relative Humidities (RH) During Spray and Storage

		Mean Virus Titre (Log_{10} $TCID_{50}$/Litre) ± SD[a]									
		EMEM					NIBS				
T	RH	0	7 mins	1 hr	2 hr	3 hr	0	7 min	1 hr	2 hr	3 hr
32°C	30%	5.3 ± 0.12	3.9 ± 0.10	2.9 ± 0.00	2.4 ± 0.06	2.2 ± 0.15	4.4 ± 0.23	4.2 ± 0.15	3.2 ± 0.12	2.5 ± 0.20	2.1 ± 0.23
	90%	5.0 ± 0.61	4.5 ± 0.60	3.7 ± 0.64	3.3 ± 0.52	2.9 ± 0.44	4.9 ± 0.20	4.2 ± 0.32	3.0 ± 0.70	2.2 ± 0.25	2.0 ± 0.10
6°C	30%	5.2 ± 0.05	3.3 ± 0.15	2.6 ± 0.12	2.4 ± 0.00	2.3 ± 0.00	4.0 ± 0.05	3.1 ± 0.15	2.9 ± 0.12	2.7 ± 0.12	2.2 ± 0.12
	90%	5.3 ± 0.00	4.8 ± 0.06	4.6 ± 0.06	4.4 ± 0.06	4.1 ± 0.12	4.3 ± 0.50	4.2 ± 0.52	3.8 ± 0.53	3.3 ± 0.65	3.0 ± 0.46

[a]SD = standard deviation

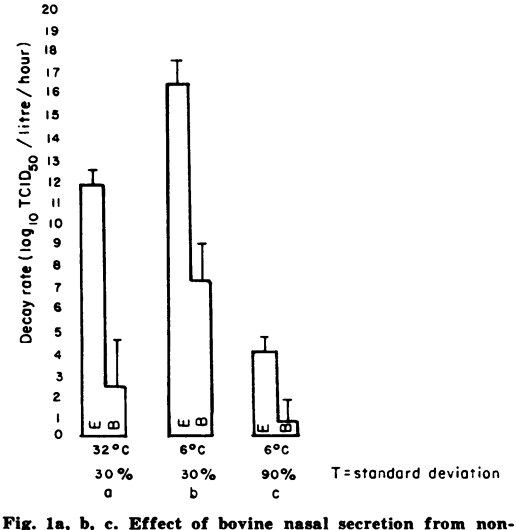

Fig. 1a, b, c. Effect of bovine nasal secretion from noninfected calf (B) and Eagle's minimum essential medium (E) on IBR virus decay rate during spray time at different temperature (32°C, 6°C) and relative humidity (30%-90%). T = standard deviation.

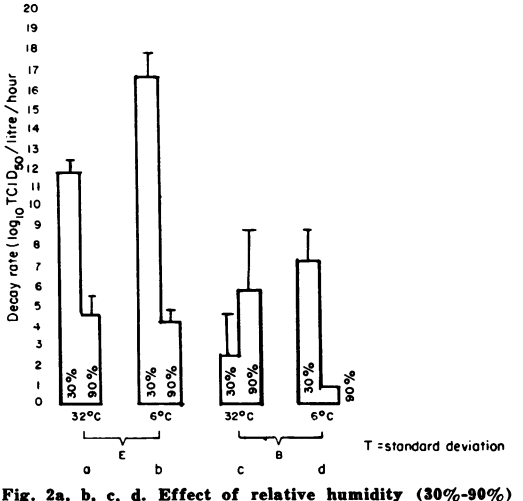

Fig. 2a, b, c, d. Effect of relative humidity (30%-90%) on IBR virus decay rate during spray in aerosols of Eagle's minimum essential medium (E) and of bovine nasal secretion from noninfected calf (B) at different temperature (32°C, 6°C). T = standard deviation.

the same medium and temperature. A significance level of 1% was used.

When the results of the above comparisons were significantly different during aging, the virus inactivation curves (regression lines) for each comparison were plotted. The regression equation used to calculate these virus inactivation curves was $y = a + b \log x$ (12) where y is the expected average virus concentration (in $\log_{10}$ $TCID_{50}$/litre), a is the point where the line crosses the y-axis (y-intercept), b is the average decay rate (regression coefficient or slope of the line) based on three replicates and log x is the logarithm to base ten of the time expressed in hours, commencing at seven minutes postspraying.

RESULTS

Particle Size of Aerosols

The PAP $\leqslant 5$ μ from NIBS and EMEM at seven minutes postgeneration are shown in Table I. At 6°C and 90% RH, the PAP $\leqslant 5$ μ from EMEM was significantly higher than from NIBS, although there was no significant difference at 30% RH. At 32°C the PAP $\leqslant 5$ μ from NIBS was significantly higher than from EMEM at 90% RH, while the opposite was true at 30% RH.

Aerosol Stability in Eagle's Minimum Essential Medium and Bovine Nasal Secretion

The concentration of the virus aerosolized and recovered at seven minutes, one, two and three hours postgeneration from aerosols of EMEM and NIBS at different RH and temperatures are recorded in Table II. Significant effects of medium, PH and temperature on the biological decay rate of IBR virus during the initial seven minutes (spraying period) are shown in Figs. 1-3. The biological decay rate was significantly higher in aerosols of EMEM than in aerosols of NIBS at high temperature and low humidity (Fig. 1a) as well as at low temperature and both levels of RH (Figs. 1b, c). At high temperature and RH the virus decay rate was not significantly different in the two media. When the role of RH was examined (Fig. 2), it was apparent that the decay rate was greater at 30% than 90% for aerosols of EMEM at both temperatures (Figs 2a, b) and for aerosols of NIBS at 6°C (Fig. 2d) while in NIBS at 32°C decay was greater at 90% RH than at 30% RH. In relation to temperature (Fig. 3), the decay rate in aerosols of both media was higher at 6°C than at 32°C at low RH (Figs 3a, b) while the contrary was true in NIBS at 90% RH (Fig. 3c).

Regression lines for the significant effects of temperature, RH and medium on decay rates between seven minutes and three hours (aging period) are shown in Figs. 4-6. The higher temperature was significantly more deleterious for the virus in both media and both levels of RH (Figs. 4a-d). The RH did not significantly influence the decay rate of the virus at 32°C in either medium, but at 6°C the virus was less stable at low humidity in EMEM (Fig. 5a) but more stable at low humidity in NIBS (Fig. 5b). The virus decay rate was greater in NIBS than in EMEM at 90% RH at 6°C and 32°C (Figs. 6a, b).

Aerosol Stability in Nasal Secretion from an Infected Calf

The concentration of the virus aerosolized and recovered at seven minutes, one, two and three hours postgeneration are given in Table III. During the spraying period the virus decay rate was greater in the nasal secretion from the infected calf than in the nasal secretion from noninfected calf at 32°C and at both RH levels (Figs. 7a, b). In an aerosol of nasal secretion from the infected calf at 32°C the 90% RH produced a higher virus decay rate than 30% RH (Fig. 8a), while the reverse was true at 6°C (Fig. 8b). The virus was also less stable in this medium at 32°C than 6°C but only at 90% RH (Fig. 9). At 6°C and 90% RH during the aging period viral inactivation was more rapid in nasal secretion from the infected calf than in nasal secretion from the noninfected calf (Fig. 10). The virus was more stable in aerosols of nasal secretion from the infected calf at 32°C than at 6°C at 90% RH (Fig. 11).

DISCUSSION

The particle size determinations showed

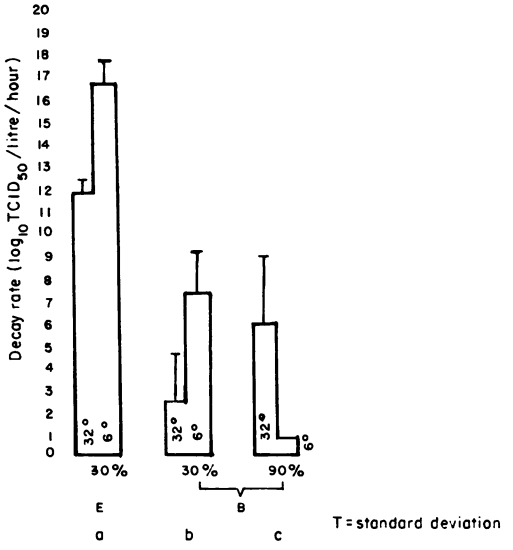

Fig. 3a, b, c. Effect of temperature (32°C, 6°C) on IBR virus decay rate during spray in aerosols of Eagle's minimum essential medium (E) and of bovine nasal secretion (B) from noninfected calf at different relative humidity (30%, 90%). T = standard deviation.

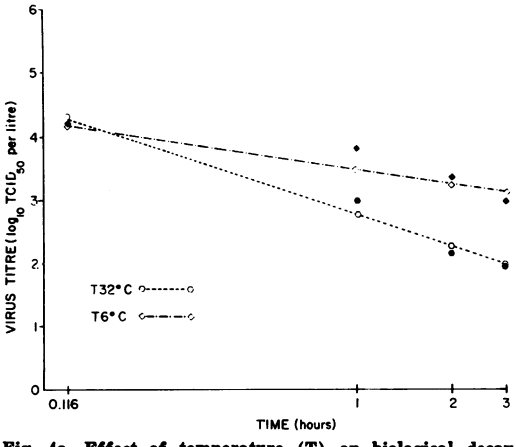

Fig. 4a. Effect of temperature (T) on biological decay of IBR virus in aerosol of bovine nasal secretion from noninfected calf at 90% relative humidity during three hour storage. ◇ ○ = average of estimated virus. ●♦ = average of observed virus titre.

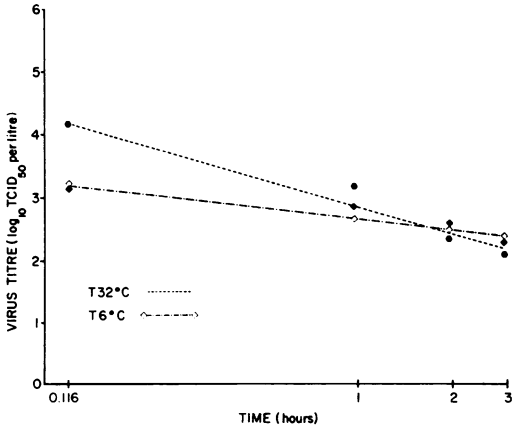

Fig. 4b. Effect of temperature (T) on biological decay of IBR virus in aerosol of bovine nasal secretion from noninfected calf at 30% relative humidity during three hour aging. ◇ ○ = average of estimated virus titre. ♦● = average of observed virus titre.

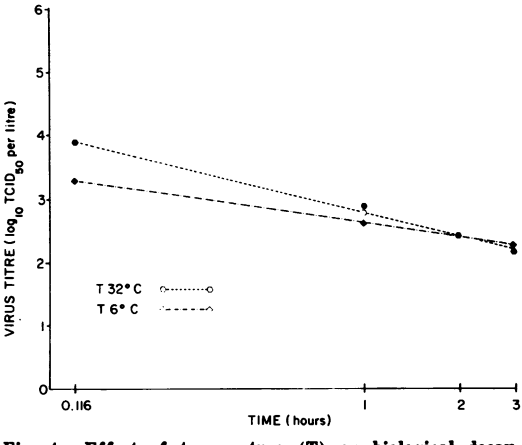

Fig. 4c. Effect of temperature (T) on biological decay of IBR virus in aerosol of Eagle's minimum essential medium at 30% relative humidity during three hour aging. ◇ ○ = average of estimated virus titre. ♦● = average of observed virus titre.

that the Devilbiss 40 nebulizer consistently produced aerosols of particles predominantly (> 88%) in the range $\leqslant 5\ \mu$, which was satisfactory for this study. Although there were some significant differences in particle size between EMEM and NIBS, these were not of great magnitude and did not appear to be responsible for the differences in virus stability in the two media which we observed.

Except for a report by Barlow and Donaldson (1) on the aerosol stability of foot and mouth disease virus, these experiments are the first to be described in which viral aerosols were generated from nasal secretion, although others have carried out aerobiological studies with viruses suspended in other "natural" media such as saliva, milk and fecal slurry (3, 4, 5). The use of nasal secretion as a suspending medium should yield more realistic data in

TABLE III. Recovery of Infectious Bovine Rhinotracheitis Virus from Aerosols of Bovine Nasal Secretion from Noninfected (BNSI) and an Infected Calf (IBS) at Different Temperatures (T) and Relative Humidities (RH) During Spray and Storage

		Mean Virus Titre ($Log_{10}TCID_{50}$/Litre) ± SD[a]									
		BNSI					IBS				
T	RH	0	7 min	1 hr	2 hr	3 hr	0	7 min	1 hr	2 hr	3 hr
32°C	30%	4.5 ± 0.20	3.8 ± 0.06	3.0 ± 0.10	2.6 ± 0.36	2.5 ± 0.21	4.7 ± 0.00	3.4 ± 0.12	2.7 ± 0.23	2.4 ± 0.26	2.3 ±0.25
	90%	4.7 ± 0.21	3.8 ± 0.45	2.6 ± 1.00	2.3 ± 0.79	1.9 ± 0.61	4.3 ± 0.52	1.8 ± 0.29	0.9 ± 0 81	0.9 ± 0.75	0.8 ± 0 69
6°C	30%	4.4 ± 0.30	3.0 ± 0.35	2.3 ± 0.72	2.1 ± 0.64	1.7 ± 0.46	4.0 ± 0.15	2.6 ± 0.31	2.2 ± 0.35	1.3 ± 0.10	0.4 ± 0.69
	90%	4.1 ± 0.15	3.7 ± 0.20	2.9 ± 0.15	2.7 ± 0.15	2.5 ± 0.06	4.1 ± 0.10	3.9 ± 0.17	0.0	0.0	0.0

[a]SD = standard deviation

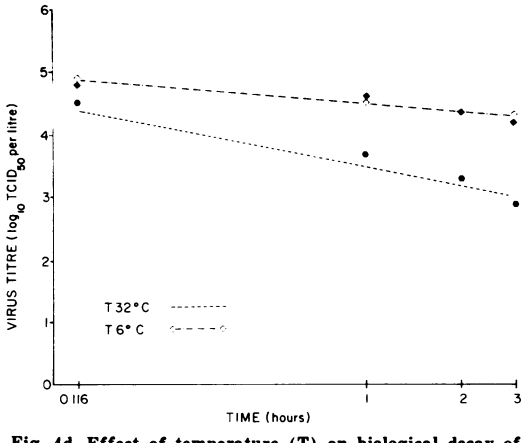

Fig. 4d. Effect of temperature (T) on biological decay of IBR virus in aerosol of Eagle's minimum essential medium at 90% relative humidity during three hour aging. ◇○ = average of estimated virus titre. ◆● = average of observed virus titre.

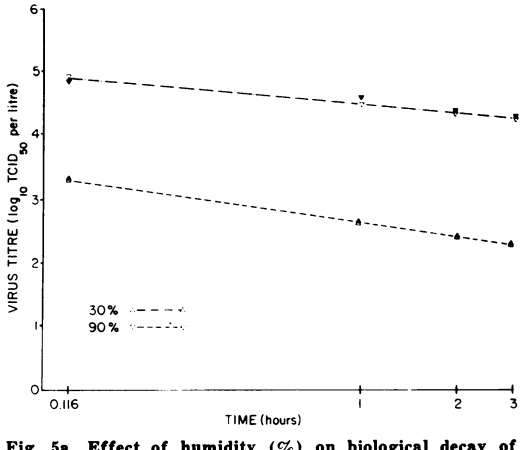

Fig. 5a. Effect of humidity (%) on biological decay of IBR virus in aerosol of Eagle's minimum essential medium at 6°C during three hour aging. ▽△ = average of estimated virus titre. ▼▲ = average of observed virus titre.

relation to viral transmission under field conditions. Clearly, the most realistic available suspending medium to use for aerobiological studies on bovine respiratory viruses would be nasal secretion from an early infection in a calf with the virus under study, but in the present study, survival of the virus in nasal secretion from an infected calf was not widely different from survival in nasal secretion from the noninfected calf, although under certain conditions survival was better in the nasal secretion from the noninfected calf. The latter differences may have been associated with minor differences in the composition of the secretion from the infected and the noninfected calf, such as the higher K+ concentration and total osmolality of the former, which could be involved in surface inactivation of the virus (15).

The results obtained for the inactivation of IBR virus in aerosols of nasal secretion suggested that this virus is able to survive well enough in the atmosphere for airborne transmission of the infection to occur, at least among intensively reared housed animals. The most favourable environmental conditions for short term survival of the virus seemed to be low temperature and high RH. The more rapid decay of IBR virus at low RH was also noted (11). Although this author used a different suspending medium at a different temperature, he found a linear relationship be-

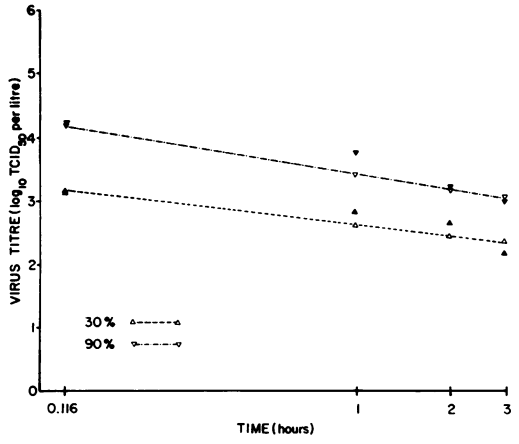

Fig. 5b. Effect of humidity (%) on biological decay of IBR virus in aerosol of bovine nasal secretion from a normal calf at 6°C during three hour aging. ▽△ = average of estimated virus titre. ▼▲ = average of observed virus titre.

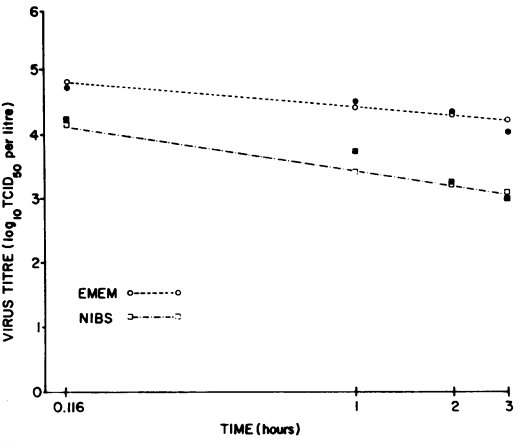

Fig. 6b. Effect of medium on biological decay of IBR virus in aerosol at 6°C and 90% relative humidity during three hour aging. ○□ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = bovine nasal secretion from noninfected calf.

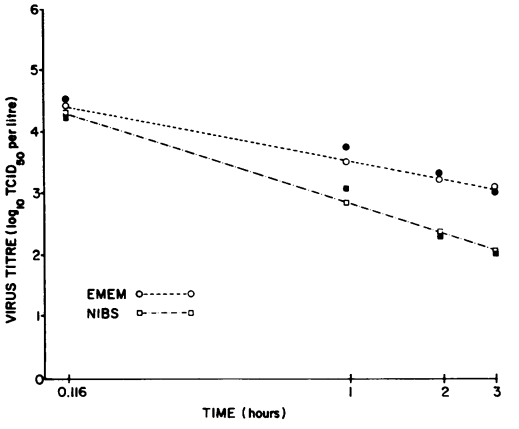

Fig. 6a. Effect of medium on biological decay of IBR virus in aerosol at 32°C and 90% relative humidity during three hour aging. ○□ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = bovine nasal secretion from noninfected calf.

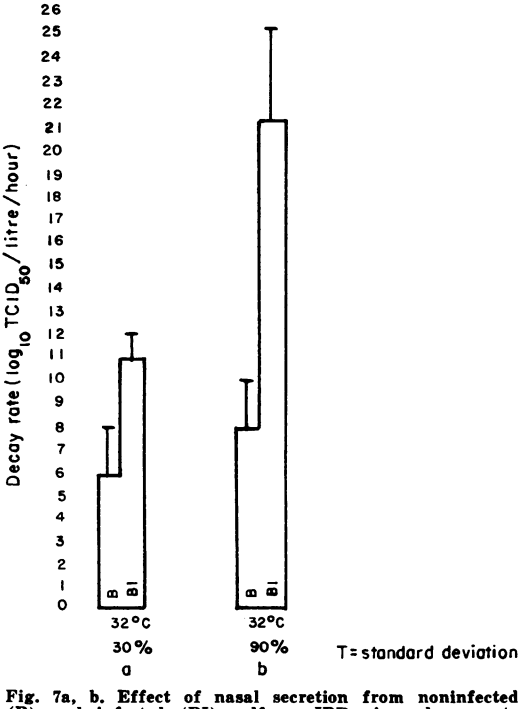

Fig. 7a, b. Effect of nasal secretion from noninfected (B) and infected (BI) calf on IBR virus decay rate during spray time at 32°C and 30%-90% relative humidity. T = standard deviation.

tween the log of viral loss and decreasing humidity. However, Donaldson and Ferris (6) showed that two strains of IBR virus were less stable at high RH and room temperature. In the present studies, the effect of temperature on the survival of the virus was most pronounced during storage of the aerosol when viral inactivation was consistently greater at the higher temperature, irrespective of media or RH. The better survival of the virus at the lower temperature during storage is in agreement with the observations made by Harper (7, 8) and Watkins *et al* (17) on vaccinia, influenza, Venezuelan equine encephalomyelitis and vesicular stomatitis virus aerosols. The high decay rate noted during spraying at low temperature and low RH may have been due to transitory freezing

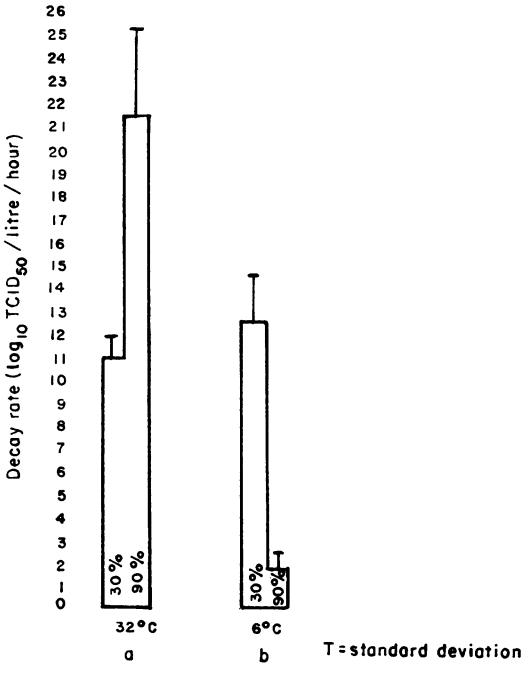

Fig. 8a, b. Effect of relative humidity (30%-90%) on IBR virus decay rate during spray in aerosol of bovine nasal secretion from an infected calf at 6°C and 32°C. T = standard deviation.

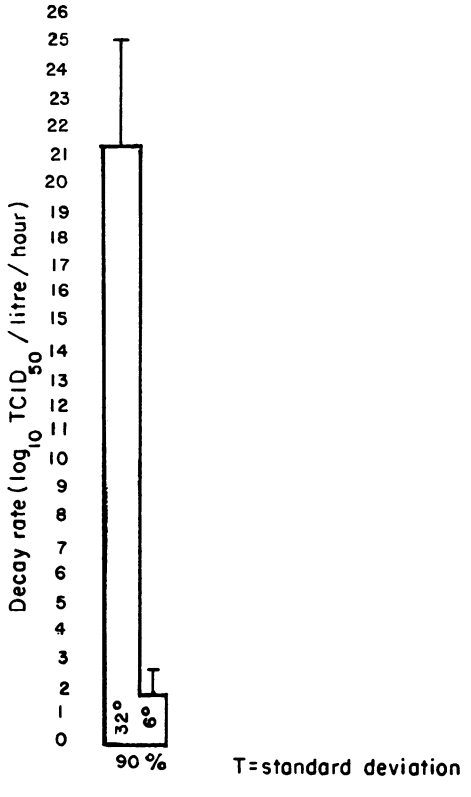

Fig. 9. Effect of temperature (32°C, 6°C) on IBR virus decay rate during spray in aerosol of bovine nasal secretion from an infected calf at 90% relative humidity. T = standard deviation.

and thawing of the virus under these conditions of evaporation which might be particularly damaging to this enveloped virus.

Infectious bovine rhinotracheitis virus was generally found to survive better during spraying from NIBS than from EMEM. The protective action of NIBS might be attributed to several factors, such as its higher viscosity, which could reduce the evaporation rate and the high protein and low salt content of NIBS compared with EMEM. Donaldson and Ferris (6) showed that IBR virus was susceptible to surface inactivation and the addition of peptone to the suspending medium was protective against surface inactivation. However, without further studies, the relative importance of the deleterious action of salts and the stabilizing effect of amino acids and glucose during aerosolization cannot be determined.

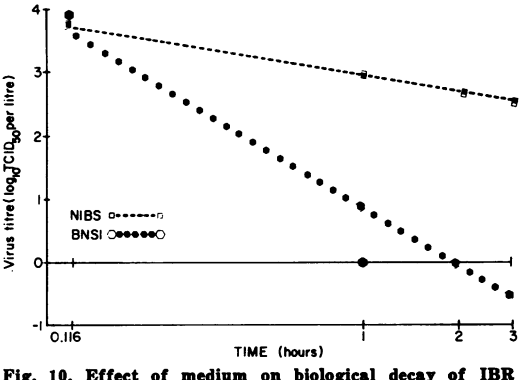

Fig. 10. Effect of medium on biological decay of IBR virus in aerosol at 6°C and 90% relative humidity during three hour aging. ○ □ = average of estimated virus titre. ● ■ = average of observed virus titre. NIBS = bovine nasal secretion from noninfected calf. BNSI = bovine nasal secretion from infected calf.

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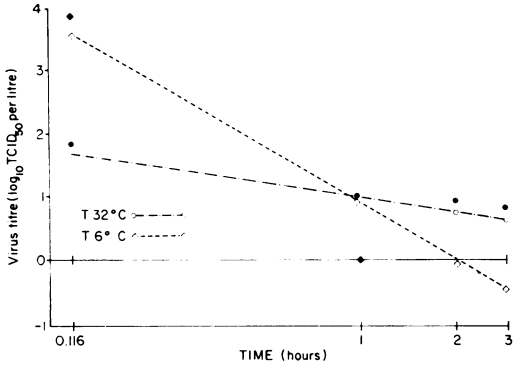

Fig. 11. Effect of temperature (T) on biological decay of IBR virus in aerosol of bovine nasal secretion from an infected calf at 90% relative humidity during three hour storage. ◇ ○ = average of estimated virus titre. ◆ ● = average of observed virus titre.

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