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SUFFIELD TECHNICAL NOTE

NO. 245

EFFECT OF RELATIVE HUMIDITY ON THE AEROSOL SURVIVAL OF SEMLIKI FOREST VIRUS DERIVED FROM SUCKLING MOUSE BRAIN (U)

by

R.A. Gaunt

DRB PROJECT NO. D-18-20-19

February 1969

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

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ABSTRACT

The effect of relative humidity (RH) on aerosols of Semliki Forest virus (SFV) is being investigated in order to provide information useful in the estimation of the hazard created if aerosols of arbor-viruses were used by an enemy in an offensive manner.

Crude extracts of SFV derived from the brains of infected three day old suckling mice are influenced by the RH when aged in the aerosol state. Survival of SFV is greatest at low RH, decreasing with increasing RH when maintained at a constant temperature.

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INTRODUCTION

It can be speculated that an enemy could use arborvirus aerosols in an offensive manner. It would be of value to determine the effect of a natural atmospheric condition such as relative humidity (RH) on the survival of a representative of the arborvirus group in order to provide information useful in the estimation of the hazard created by such an eventuality.

Semliki Forest virus (SFV) is a member of the Group A arborviruses (Casals 1961), first isolated in Uganda (Smithburn and Haddow 1944). Faulker and McGee-Russell (1968) determined the complete virion to be 70-80 nm in diameter with a core about 50 nm in diameter. SFV agglutinates goose erythrocytes and contains infectious ribonucleic acid (Cheng 1958).

SFV infection is ordinarily mediated through a mosquito vector, however, infection of young adult syrian hamsters (Henderson *et al* 1967) and young adult field mice (Peromyscus maniculatus) (Gaunt - unpublished data) has been accomplished with aerosols.

SFV was propagated in three different host systems, the whole animal (mouse), the embryo (embryonated hens' eggs) and an incomplete system (chicken embryo tissue culture) in order to determine whether the inherent aerosol stability would vary under changes of RH by virtue of the system in which the virus was propagated.

The effects of RH on SFV derived from embryonated eggs and/or chicken embryo tissue culture will be presented in subsequent reports.

MATERIALS AND METHODS

Virus

SFV (original strain) was obtained from the American Type Culture Collection, virus repository VR 67. At the time of receipt, it had been passaged eleven times in mouse brain and exhibited a titre of $1 \times 10^{7.6}$ LD_{50}/ml in terms of adult mice by intracranial inoculation.

The crude virus stock for use in these aerosol decay studies was produced using the following protocol: Fifty 3-day old suckling mice were inoculated intracranially with a 0.02 ml suspension of the above virus diluted to exhibit a titre of 1 x 10^4 plaque forming units (PFU)/ml. At approximately 28 hours (Gaunt 1968), all mice were sacrificed. The mice were decapitated, brain tissue aseptically removed and prepared as a 10 per cent suspension in Hanks' balanced salt solution (HBSS) containing 100 units/ml penicillin and streptomycin and 0.75 per cent bovine plasma albumin (BPA). To dissociate virus from brain tissue, the suspension was homogenized in a Waring blender (cooled to 4°C with ice) for 3 minutes with one minute rest period to minimize frothing. Cellular debris was removed by centrifugation in the PR2 International Centrifuge at 1000 rpm for 10 minutes at 4°C. Protamine sulfate (salamine) at a concentration of 5 mg/ml was added to the preparation, which was left at 4°C overnight in order to precipitate non-infectious protein impurities (Cheng 1961). The preparation was centrifuged again as above. The crude virus stock was dispensed in 1.25 ml amounts into 7 x 100 ml tubes, flame sealed and stored immediately at -70°C. The crude virus preparation of SFV exhibited a titre of 1 X $10^{9.7}$PFU/ml.

Aerosol Procedures

The aerosol holding facility was a 500 liter (L) stainless steel rotating (3-4 rpm) drum (Goldberg et al 1958) contained within a temperature and humidity controlled insulated chamber.

The temperature and humidity within the drum was controlled by the addition of a chemically free, filtered secondary air supply.

Upon equilibration of the aerosol holding facility at the selected temperature and relative humidity, the virus preparation, consisting of 1.0 ml of crude virus stock added to 19.0 ml of phosphate buffered saline (PBS) pH 7.0 (titre 1 x $10^{8.4}$PFU/ml), was atomized into the drum by way of a mixing chamber using a modification of the Collison atomizer operated at 26 pounds per square inch pressure for 10 minutes. The drum was then isolated from further air disturbance by check valves.

One minute aerosol samples were obtained every hour for 5 hours after dissemination, using the 12-30 all glass impinger (AGI) (CHATAS Glass Co., Vineland, N.H., U.S.A.) containing 20 ml of PBS.

Virus Assay

The virus content of the impinger fluid used to sample the aged aerosol was assayed on monolayers of primary chicken embryo cells overlayed with equal parts instant powdered tissue culture medium combining Eagles's Minimal essential medium and Earle's balanced salt solution (Grand Island Biological Company, Grand Island, N.Y., U.S.A.) and 2 per cent agarose (SeaKem Bausch and Lomb). Impinger fluid was decimally diluted in HBSS containing 100 units/ml penicillin and streptomycin plus 0.75 per cent BPA. 0.3 ml of each dilution was added to duplicate monolayers and plaque counts were the average of the duplicate assays.

Dissemination and Assay of Physical Tracer

In order to differentiate between biological and physical decay of aged SFV aerosols, sodium fluorescein dye was used as a tracer. It was found that the dye could not be mixed directly into the virus material to be atomized because it tended to give erratic results with regard to virus decay. Therefore, several experiments were done at 20, 50 and 80 per cent RH using dye alone in order to determine the physical decay of the aerosol at a particular RH. A standard curve was produced which related the concentration of sodium fluorscein in impinger fluids with arbitrary fluorescent units which were assayed using the Aminco-Bowman 4-8106 spectrophotofluorometer.

Sodium fluorescein at a concentration of 10 μg/ml was disseminated into the 500L drum for 10 minutes. The aerosol was sampled for one minute every hour for five hours, using the 12-30 AGI containing 20 ml of PBS pH 7.0. The concentration of the dye in the impinger fluid of the hourly samples was related to the concentration of dye in the impinger fluid at time zero.

RESULTS

Figure 1 illustrates the effect of RH on mouse brain extracts of SFV over a 5-hour period at low, intermediate and high RH and a constant temperature of 21°C. Results presented for each RH level were the arithmetical mean of between 5 and 7 experiments.

Figure 2 illustrates the effect of RH on the 5-hour aged aerosol of SFV.

Figure 3 indicates the per cent physical decay of the sodium fluorescein tracer over a 5-hour period.

DISCUSSION

Mouse brain extracts of SFV when aged in the airborne state are most susceptable to biological decay at high RH and least susceptable at low RH when aerosols are kept at a constant temperature of 21°C; a characteristic which appears to be shared by other ether sensitive viruses and in contrast to some ether stable viruses which tend to survive better at high RH (Harper 1961), (Harper 1963), (Watkins *et al* 1963) and (Akers *et al* 1966). Most of the inactivation would appear to take place within the first hour after dissemination and, in fact, within the first few minutes, because the "zero" sample has a mean age of 5½ minutes, at which time a noticeable drop in infectivity was observed in the case of the 80 per cent RH aerosol and, to a lesser degree, in the 50 per cent and 20 per cent RH aerosols, respectively.

Sodium fluorescein proved most effective as a physical decay tracer. Loss of aerosol due to physical decay was essentially nil. Loss of infectivity in dissemination fluids due to the refluxing action of the atomizers was negligible and, in fact, was sometimes slightly elevated.

REFERENCES

Akers, T.G., Bond, S. and Goldberg,L.J. 1966 "Effect of Temperature and Relative Humidity on Survival of Airborne Columbia SK Group Viruses" App. Microbiol. 14, 361.

Casals, J. 1961 Bulletin of the World Health Organization, No. 24.

Cheng, P-Y 1958 "Infectivity of Ribonucleic Acid From Mouse Brains Infected With Semliki Forest Virus" Nature 181, 1800.

Cheng, P-Y 1961 "Purification, Size, Morphology of A Mosquito-borne Animal Virus Semliki Forest Virus" Virology 14, 470.

Faulkner, P. and S.M. McGee-Russell 1968 "Purification and Structure of Semliki Forest Virus Isolated From Mouse Brain" Can. J. Microbiol. 14, 153.

Gaunt, R.A. 1968 "Propagation and Assay of Semliki Forest Virus" Suffield Technical Note No. 220.

Goldberg, L.J., Watkins, H.M.S., Boerke, E.E. and Chatigny, M.A. 1958 "The Use of A Rotating Drum For The Study of Aerosols Over Extended Periods of Time" Am. J. Hyg. 68, 85.

Harper, G.J. 1961 "Airborne Microorganisms: Survival Tests With Four Viruses" J. Hyg. 59, 479.

Harper, G.J. 1963 "Some Observations on The Influence of Suspending Fluids on The Survival of Airborne Viruses" Proc. 1st Internat. Symp. on Aerobiol., pp 335-341.

Henderson, D.W., Peacock S. and Randles, W.J. 1967 "On The Pathogenesis of Semliki Forest Virus (SFV) Infection in the Hamster: Brit. J. of Expt'l. Pathol. XLVIII, 228.

Smithburn, K.C. and Haddow, A.J. 1944 "Semliki Forest Virus. I. Isolation and Pathogenic Properties" J. Immunol. 49, 141.

Watkins, H.M.S., Goldberg, L.J., Deig, E.F. and Leif, W.R. 1963 "Behaviour of Colorado Tick Fever, Vesicular Stomatitis, Neurovaccinia and Encephalomyocarditis Viruses in The Airborne State" Proc. 1st Internat. Symp. on Aerobiol., p 381.

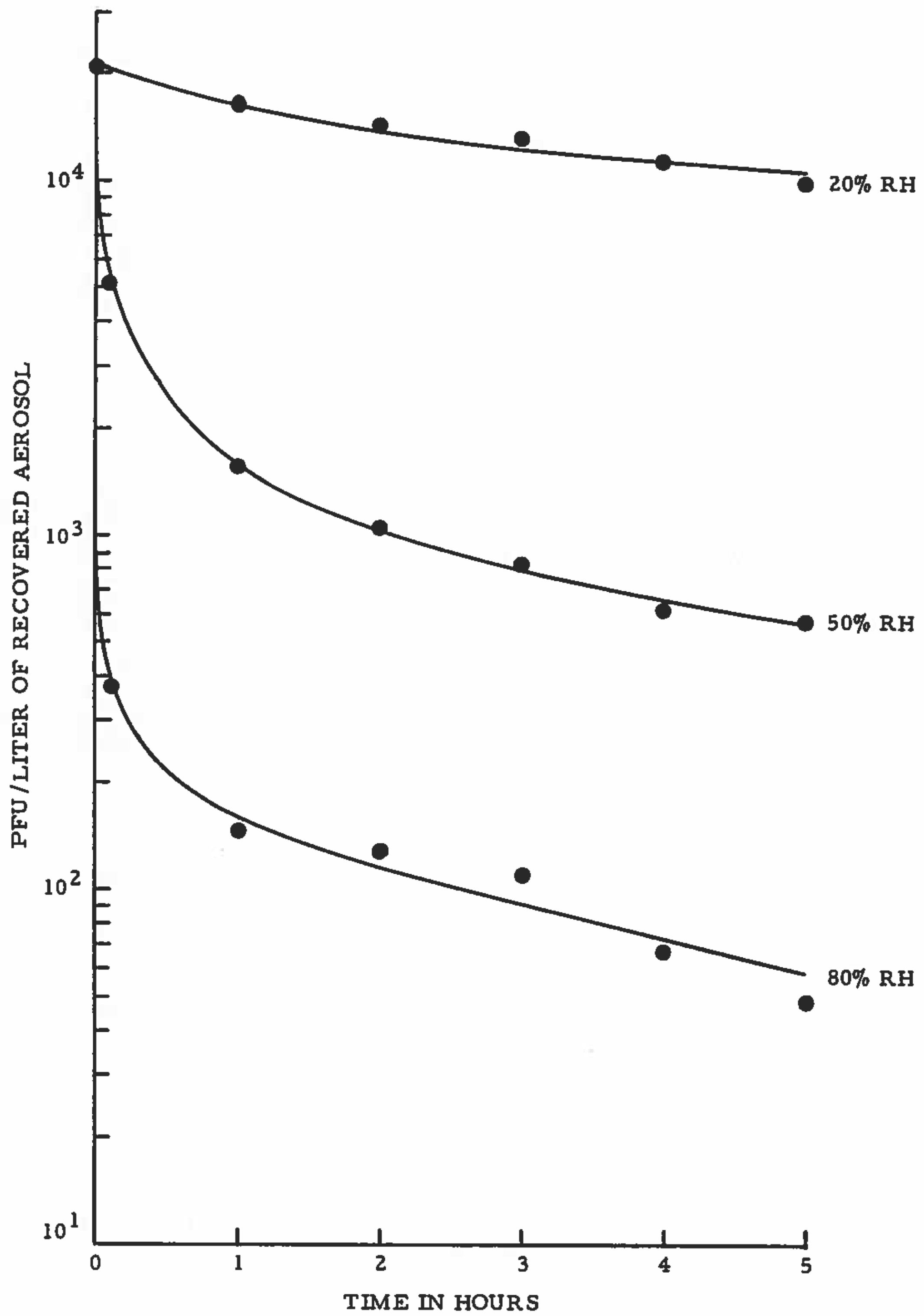

FIG. 1 - Effect of Relative Humidity on the Biological Decay of Aerosols of Mouse-Brain Extracts of Semliki Forest Virus

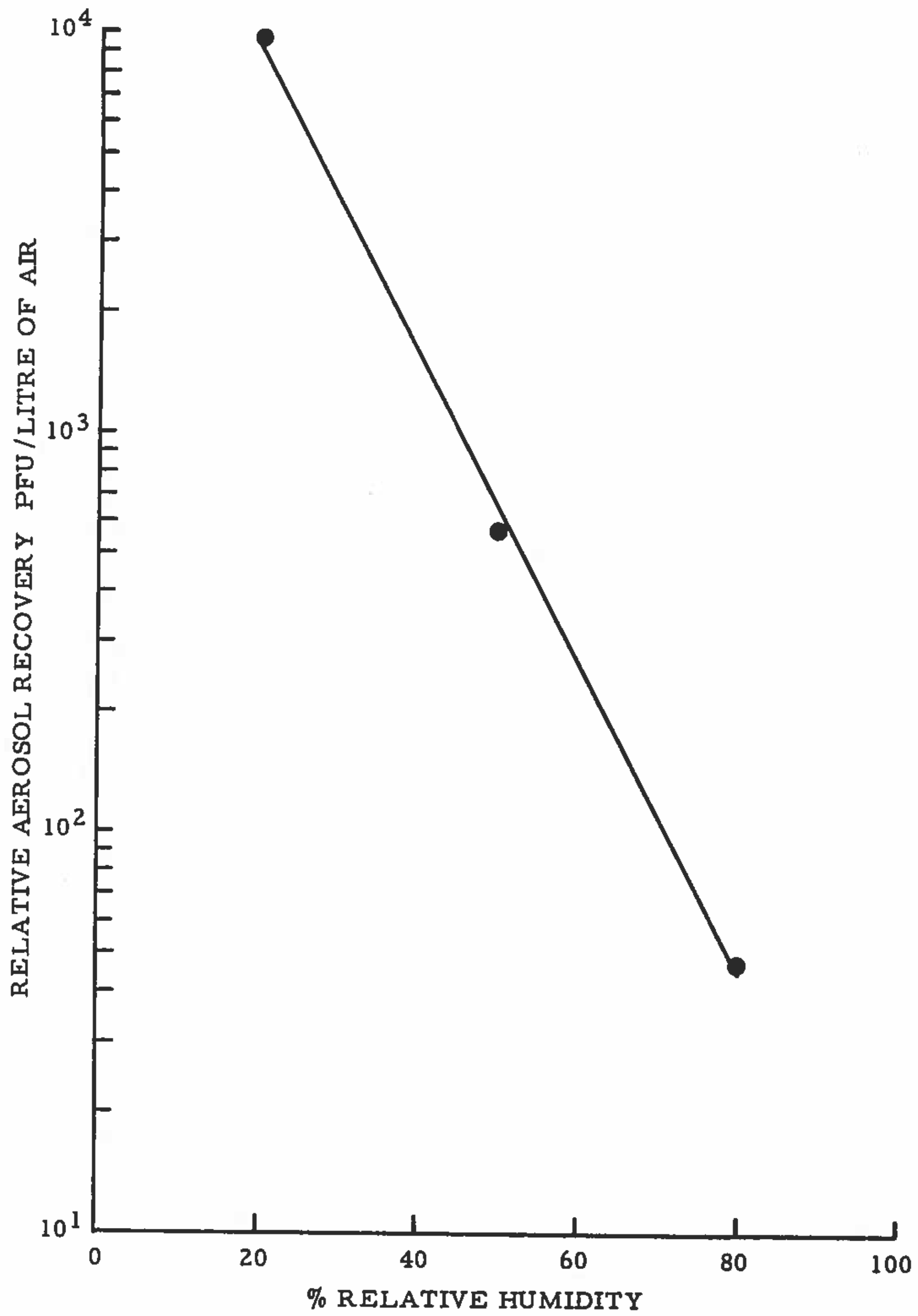

FIG. 2 - Effect of Relative Humidity on Semliki Forest Virus Aerosols At Constant Temperature 21°C Aerosol Age 5 Hours

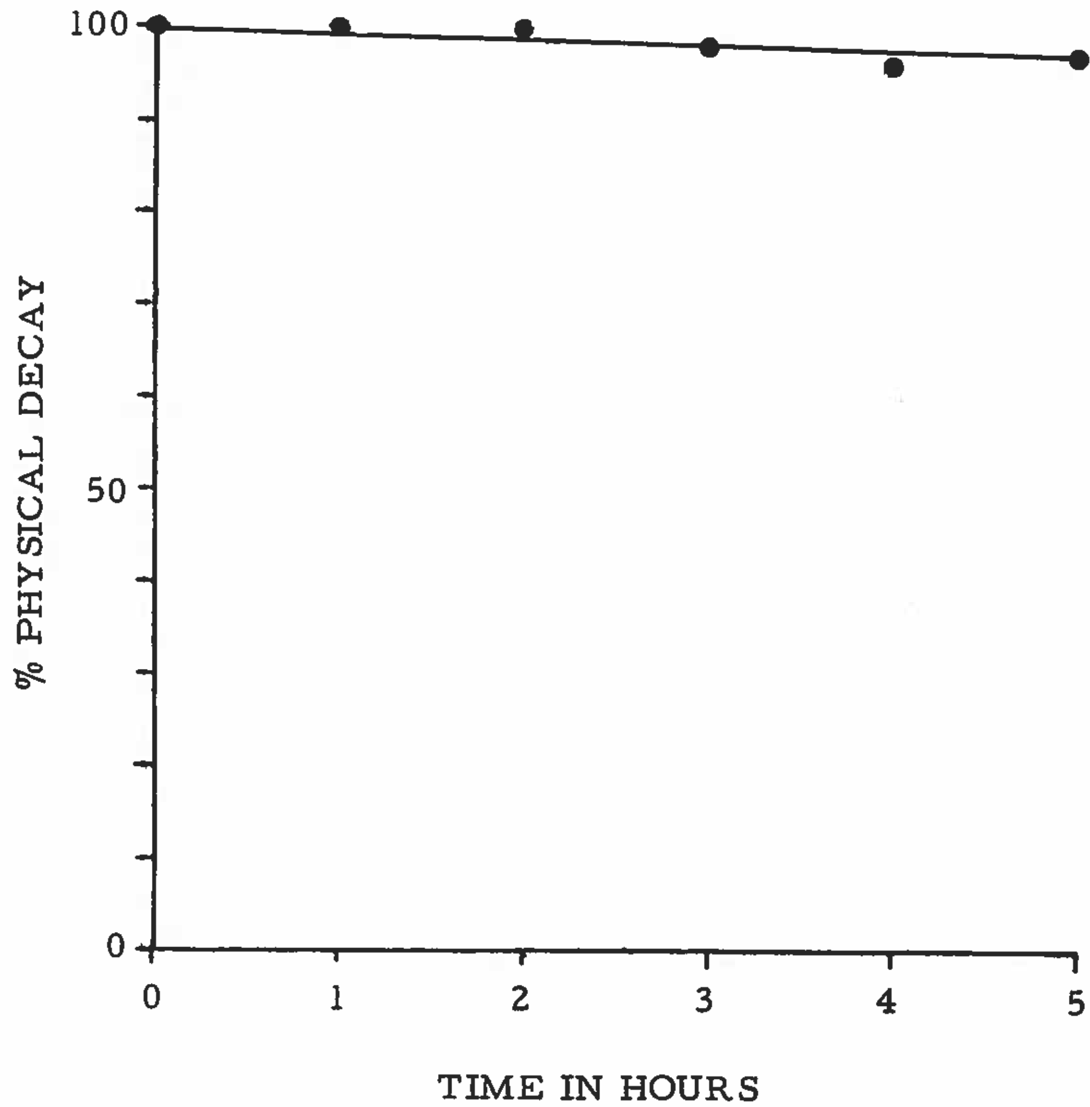

FIG. 3 - Physical Decay of Sodium Fluorescein Tracer Over 5 Hour Period

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1. Aerosol Decay 2. GRP A Arborvirus

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