Stability of St. Louis Encephalitis Virus in the Airborne State

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The aerosol stability of St. Louis encephalitis (SLE) virus was studied over a 6-hr period at a temperature of 21 C and relative humidity values of 23, 46, 60, and 80%. Aerosols were generated from and collected in 0.75% bovine albumin-buffered saline, and spores of Bacillus subtilis var. niger were used as the tracer to determine the physical decay of the aerosols. Aerosol samples were titrated in BHK-21 cell monolayers for surviving SLE virus. The results of this study indicated that, under the test conditions employed, relative humidity had no influence on the stability of SLE virus in the airborne state.

In a survey of laboratory-acquired arbovirus infections in 38 countries, Hanson et al. (3) found that approximately 17% of the infections were probably acquired by the airborne route. This suggested the desirability of studying the aerosol stability of certain arboviruses to determine under what conditions they would represent potential health hazards for laboratory personnel. The present study was concerned with the effects of relative humidity at 21 C on the stability of airborne St. Louis encephalitis (SLE) virus.

MATERIALS AND METHODS

Virus. The Parton strain of SLE virus was used in this study. The virus was obtained as the fourth suckling mouse passage from the Rockefeller Foundation and we passed it five additional times. A 20% suckling mouse brain suspension was prepared as the working stock and was stored at -65 C. This working stock of SLE virus had a TCID50 of 10^4.4/ml when titrated in baby hamster kidney (BHK-21) cells.

Viral assays. The established, fibroblast-like cell line, BHK-21, was employed for all viral assays. The growth medium used was 90% Eagle basal medium, 10% calf serum, and 50 units of penicillin, 5 μg each of streptomycin and bacitracin, and 25 μg of neomycin per ml of medium. Maintenance medium consisted of 97% Eagle basal medium and 3% calf serum with the antibiotics as stated. During the assay of SLE virus, the maintenance medium was changed daily. The procedures of Karabatsos and Buckley (6) were employed for the growth, maintenance, and storage of BHK-21 cells, except that a bicarbonate buffer was used instead of a TRIZMA buffer.

Initial virus titrations involving 10-fold dilutions and six tubes per dilution were used to establish approximate median end points by use of the Weibull mortality grid (10). More precise titrations were later based on three-fold dilutions and 16 tubes per dilution. TCID50 values were calculated by the method of Reed and Muench (8).

Physical tracer. Spores of Bacillus subtilis var. niger (Bacillus globigii) were used to determine physical decay rates and recovery ratios of aerosols. A working stock suspension containing 5.5 × 10^6 spores/ml was prepared in 0.75% bovine albumin-borate saline (BABS), pH 7.4.

Aerosol generation, storage, and sampling. An environmental safety cabinet housed the equipment used for generation, storage, and collection of aerosols. The characteristics and operation of the safety cabinet and storage drum, along with an explanation of the control of temperature and relative humidity, were described by Kelley (Ph.D. Dissertation, University of Arizona, Tucson, 1967). Aerosols were generated from a De Vilbiss no. 40 nebulizer with a compressed air supply of 25 lb/in². The spray suspension was prepared by mixing 4.2 ml of working spore suspension and 0.6 ml of SLE virus stock. This mixture was sprayed for 4 min at a total air flow rate of 250 liters/min.

A stainless-steel rotating drum chamber (2) having an 1,100-liter capacity was used to store the aerosols. The drum was rotated at 3.5 rev/min during all phases of the operation. After completion of each experiment, the drum was air-washed at a rate of 250 liters/min for a minimum of 20 min.

One-minute aerosol samples (12.5 liters) were obtained with sterile, all-glass Impingers (AGI-30; reference 1) containing 20 ml of 0.75% BABS and two drops of Dow-Corning antifoam A.

Experimental procedures. Aerosols were generated at 21 C and at relative humidity values of approximately 80, 60, 46, and 30%. Samples were taken 15 min after atomization and at hourly intervals for 6 hr. Two trials were conducted at each relative humidity value. Spray and impinger samples for viral assays were stored at -65 C until cell cultures were available. The spore content of each sample was determined promptly after collection by preparing 10-fold dilutions in distilled water and plating 0.1-ml quantities.
three plates per dilution, on Tryptic Soy Agar (Difco). The plates were incubated at 37°C for 24 hr. Dilutions of spore suspensions yielding less than 300 colonies were counted and decay slopes were estimated by the method of least squares.

**Virus neutralization tests**. Neutralization tests were performed in BHK-21 cell cultures by using essentially the procedures described by Schmidt (9). Hyperimmune rabbit serum was prepared with a previous passage of the same SLE virus as was used in the aerosol experiments.

**RESULTS**

The results of spore assay (Table 1) were used to estimate the total physical decay of the aerosols and the biological decay of the virus aerosols. The slope of the physical decay at each trial was found to be insignificant at the 95% confidence level. From the average of the slopes, it was determined that the approximate half-life of the spores in the aerosol state was 21.5 hr.

It was anticipated that appreciable virus inactivation would occur at some point during the 6-hr storage. Accordingly, an assay for virus infectivity was performed at each sampling point of the trials conducted at relative humidity values of 78 and 80%. Results of this preliminary assay (Table 2) indicated that the viral aerosols were stable during the 6-hr storage period. It was therefore decided to assay the 0.25- and 6-hr samples of one trial at each of the other relative humidity values to obtain a general, overall estimate of the virus aerosol stability. The results of these assays (Table 2) indicate no appreciable loss of infectivity at any relative humidity value.

More precise assays, with threefold dilutions and 16 tubes per dilution, were performed on the 0.25-hr and the 6-hr samples for each of the eight trials. The results of these assays (Table 3) revealed no appreciable changes in virus concentration. Evaluation of these data by analysis of variance confirmed these observations and established that, at the 95% confidence level, relative humidity had no significant effect on aerosolized SLE virus. However, statistically significant differences in concentration were found to exist between sampling points, that is, between 0.25 and 6 hr of storage. The total decay of the aerosolized virus was obtained from the average of the indi-

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**Table 1. Total plate counts of spores of B. subtilis var. niger recovered from samples of stored aerosols sprayed from a mixture of spores and St. Louis encephalitis virus**

<table>
<thead>
<tr>
<th>Per cent relative humidity</th>
<th>Storage period (hr)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.25 1 2 3 4 5 6</td>
</tr>
<tr>
<td>78</td>
<td>82 79 72 69 81 76 86</td>
</tr>
<tr>
<td>80</td>
<td>80 102 97 63 84 65 65</td>
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<tr>
<td>61</td>
<td>76 94 82 51 68 60 68</td>
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<tr>
<td>60</td>
<td>83 74 65 78 76 71 64</td>
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<td>46</td>
<td>84 75 64 62 66 79 73</td>
</tr>
<tr>
<td>46</td>
<td>65 73 76 65 62 69 78</td>
</tr>
<tr>
<td>35</td>
<td>93 93 79 77 84 81 73</td>
</tr>
<tr>
<td>23</td>
<td>104 106 103 97 71 109 92</td>
</tr>
</tbody>
</table>

* Each value represents a total of three plates. Each plate was inoculated with 0.1 ml of a 10⁻¹ dilution of impinger fluid.
vidual decay constants for each trial. Assuming an exponential function of decay for the aerosolized virus, the average total decay rate of 0.1932 changes per hr was equivalent to a half-life of 2.6 hr. When corrected by subtracting the rates of decay attributable to sampling ventilation and settling, a rate of 0.1610 changes per hr (4.3 hr half-life) was obtained.

Tube culture neutralization tests confirmed the identity of the stock virus preparation of SLE virus. Moreover, it was established that the cytopathic effect induced in cell cultures by the 6-hr samples was prevented by SLE immune serum. This, along with controls indicating that spore suspensions and imprinter spore preparations did not cause cytopathic effect in BHK-21 cells, confirmed the specificity of the cytopathic effect observed in the titrations of the various aerosol samples.

**DISCUSSION**

Previous stability studies by others with selected arboviruses have indicated a general pattern of increased survival at either low or high relative humidity. Harper (4) found that Venezuelan equine encephalomyelitis virus survived best at low humidity, whereas Miller and others (7) found that high humidity favored the stability of yellow fever virus and Rift Valley fever virus. Watkins et al. (12) found that Colorado tick fever virus survived best at high relative humidity; vesicular stomatitis virus, considered by some to be an arbovirus, was adversely affected at high humidity. Songer (11) reported that vesicular stomatitis virus survives best at low relative humidity.

The results of our study indicate that, under the test conditions employed, relative humidity had no influence upon the stability of SLE virus in the airborne state. Whether this stability is an inherent property of the virus or is due to the protective properties of the suspending medium has not been established by this investigation. Harper (5) reported that the influence of relative humidity on the survival of airborne viruses may be modified by certain ingredients in the suspending medium. In the studies of other workers cited earlier in this discussion, the lack of uniformity in the suspending media for spraying and collecting of aerosols adds to the difficulty in attempting to form generalizations about aerosol stability of arboviruses. Because of this lack of standardization, it is impossible to compare, in absolute terms, the findings of this study with previously reported results of stability studies involving other arboviruses. However, our results do appear to be unique in that they suggest that SLE virus may be stable over a considerably broader range of humidity than would be anticipated from the results reported with other arboviruses.

In terms of the original objectives of this study, it appears that airborne SLE virus atomized from 0.75% BABS, a fairly commonly used suspending medium for arboviruses, forms stable aerosols that represent a potential health hazard for the laboratory personnel working with this virus, and this stability is independent of relative humidity.

We are not suggesting, however, that airborne infection is of importance in the transmission of SLE virus in nature.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**