Airborne Stability of Simian Virus 40

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The influence of relative humidity on the airborne survival of simian virus 40 (SV40) was studied by allowing virus aerosols to age in rotating drums at 21 or 32 C and at a relative humidity (RH) value ranging from 22 to 88%. Airborne SV40 virus was stable at every RH tested at 21 C, but aerosols maintained at 32 C were inactivated within 60 min at mid-range RH values. The unusual stability at 21 C over a broad RH range indicates that potentially biohazardous situations may occur under laboratory conditions if this virus becomes accidentally airborne.

The estimated potential of biohazards from airborne viruses has been increased by recent findings about the airborne infectivity of viruses or their nucleic acids. Infectious viral nucleic acids, for instance, have been shown to exhibit even greater aerosol stability than the intact virions from which they were extracted (3, 5, 7); alteration of the protein coat at certain humidities was suggested as an important factor influencing virus survival in the airborne state. Relatedly, Benbough (4) recently reported that inactivation of airborne poliovirus could be reversed when prehumidification was employed prior to aerosol sampling. For this reason, control measures which may appear to be effective for virus inactivation when sampling is done by established techniques may, in fact, ameliorate survival. We decided to study the airborne stability of simian virus 40 (SV40) virus, and to examine the potential of relative humidity (RH) manipulation as a means of biohazard control.

MATERIALS AND METHODS

Virus. A single lot of the Rh-911 strain of SV40 virus was prepared in primary African green monkey kidney (AGMK) cell monolayers maintained in Eagle basal medium (BME) supplemented with 2% fetal bovine serum. The infected tissue culture fluid was centrifuged to remove cellular debris, and the resulting supernatant fluid was titered 10^8 50% mean tissue culture infectious doses ($TCID_{50}$) per ml. Samples were stored at −70 C until employed for aerosol experiments.

Aerosol equipment. Aerosols were aged in a 500-liter rotating drum (9) maintained at a holding temperature of either 21 or 32 C with RH values ranging from 22 to 88%. SV40 virus aerosols were generated using a Collison 3-jet atomizer operated for 1 min under standard conditions which produced airborne particles with a mean diameter of 2 μm.

Antifoam (Dow-Corning Antifoam B) was added

(final concentration of 1%) to the viral suspension to prevent excess foaming during aerosolization. Following the 1-min aerosolization and fill time, the drum was allowed to equilibrate (for uniform aerosol distribution within the drum) for an additional 5 min prior to initial sampling.

Aerosol sampling. Aerosol samples were collected at 5, 10, 15, 30, and 60 min after drum equilibration. Samples were collected using an AGI-30 Sampler (12) operated for 1 min. The sampler fluid consisted of 20.5 ml of Eagle minimal essential medium (MEM) plus 1% antifoam.

Virus assay. Serial 10-fold dilutions were made of atomizer and impinger fluid samples. Amounts of 0.1 ml of each sample dilution were innoculated into four AGMK culture tubes. Inoculated cultures were maintained with MEM supplemented with 2% fetal bovine serum and incubated at 37 C in stationary racks. Fresh medium was added to cultures on days, 4, 9, and 15 postinoculation. Cultures were examined for cytopathic effects through 21 days. The method of Reed and Muench (11) was used for $TCID_{50}$ calculations. Airborne virus concentration was expressed as $TCID_{50}$ per milliliter of impinger fluid. Survival patterns were plotted using the initial 5-min sample as the first point rather than the actual zero time because the exactness of the curve prior to this time could not be determined in these experiments.

RESULTS

In twelve experiments SV40 titers remained constant (8.0 ± 0.2 $TCID_{50}$) during and after aerosolization, i.e., the physical stresses of atomization were not deleterious to virus in liquid suspension. Based on known delivery rates the theoretical virus aerosol concentration was similar to the actual drum concentrations observed. There was little, if any, inactivation attributed to the mixing of aerosolized virus with secondary air or during equilibration of the aerosol in the rotating drums. Accordingly, that SV40 virus which was aerosolized was very stable at 21 C (Fig. 1). Comparison of recoveries
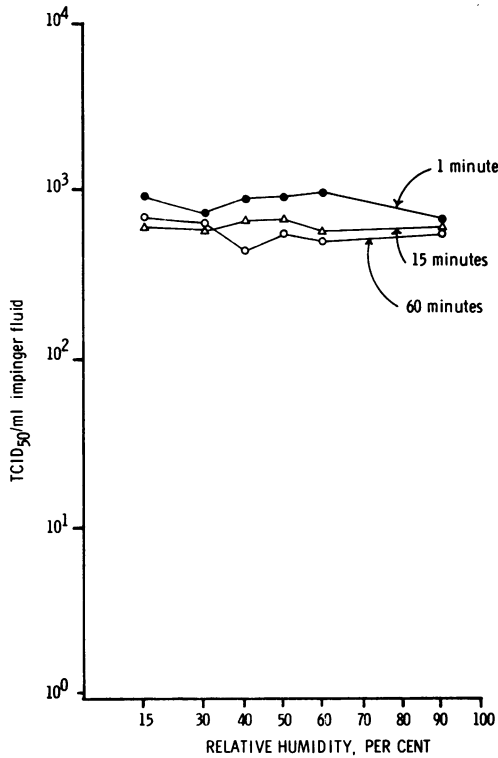
FIG. 1. *Recovery of airborne SV40 from aerosols aged at 21 C at different RH values.*

at 1 min and 60 min showed a decay rate of 0.7% per min.

Increasing the holding temperature to 32 C did not significantly alter recovery of infectious SV40 virus from 1-min aged aerosols (Fig. 2). However, after 15 min at 32 C, a marked mid-range RH sensitivity became evident, such that by 30 min no virus recovery was noted from samples aged at 60% RH. After 60 min, at both 50 and 60% RH, impinger samples were negative. Based on 1-min and 15-min virus recoveries, the decay rate was determined to be approximately 6% per min. It was evident that SV40 virus aerosols exhibited mid-range RH-dependent inactivation of 32 C.

DISCUSSION

Larson et al. (10) reported that Rauscher leukemia virus (RLV) was rapidly inactivated (99%) in the process of aerosolization and equilibration in a static cloud chamber conditioned at 24 C and 35% RH. Thereafter, the remaining airborne virus continued to remain infectious for at least 35 min. This led the authors to conclude that RLV was relatively stable (decaying at approximately 1.0% per min) under the environmental conditions studied. Based on the airborne decay rate noted for SV40 virus at

21 C, it appears that airborne SV40 and RLV may have similar airborne stability characteristics.

Webb(14) reported that the airborne stability of Rous sarcoma virus (RSV) aerosols was dependent on salt, protein, or polyhydroxyl-type compounds used in virus-suspending media. For instance, RSV survived at RH values greater than 60% when suspended in distilled water. When the virus was suspended in citrate buffer prior to aerosolization, it survived best at RH values below 40%. This points out some of the difficulties encountered in attempting to derive effective and universal biohazard control measures by manipulating only RH. Such RH control measures would not be applicable to airborne SV40 virus.

Watkins et al. (13) showed that survival of airborne vesicular stomatitis virus (VSV) decreased with successive increases in temperature, although the RH at which maximum airborne inactivation occurred remained constant. Airborne EMC group viruses also exhibited a critical RH-dependence that did not change as a function of temperature (1). EMC aerosols maintained at 16 or 32 C also exhibited mid-range inactivation patterns which were established immediately post-aerosolization and which persisted with no significant changes
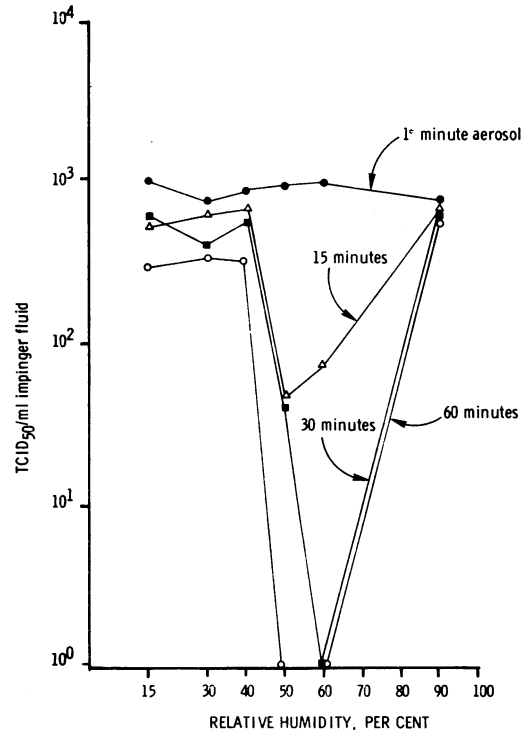

FIG. 2. *Effect of RH on survival of airborne SV40 virus at 32 C.*

(aside from actual rates of inactivation) throughout the 6-h study period. Aerosols of SV40 did not exhibit RH-dependent inactivation patterns independent of temperature as observed with these two viruses.

Ehrlich and Miller (8) reported that Venezuelan equine encephalomyelitis virus aerosolized from a liquid suspension yielded similar aerosol recoveries and inactivation rates within the RH range of 18 to 90% and at temperatures from −40 to 24 C. At 49 C, a 10-fold increase in biological decay occurred. However, RH values from 70 to 90% were most detrimental to survival at this temperature. Like airborne Venezuelan equine encephalomyelitis virus, SV40 aerosols exhibited high temperature RH-dependent inactivation patterns differing only, in this case, by different RH values.

Our findings with SV40 virus aerosols indicate that temperature has an important effect on airborne survival. Previous studies, which have revealed that infectious viral nucleic acids were not damaged with respect to biological activity either by atomization, aerosol storage, or collection (3), indicated that airborne inactivation of SV40 virus at 32 C must be associated primarily with damage to the protein component of the virion (conformational changes) which, in turn, caused a change in moiety or collapse of the SV40 deoxyribonucleic acid (DNA) molecule. More than likely, however, the SV40 DNA retained its infectivity. Although not ascertained in the study reported herein, the recent report of de Jong et al. (6) supports this conclusion. The authors noted that infectivity of ribonucleic acid (RNA) extracted from intact virions collected from poliovirus aerosols was stable at RH values deleterious to whole virus survival. This, coupled with the finding of Akers et al. (2) that aerosols of infectious EMC-viral RNA could infect mice, may point to another aspect of the biohazard potentials for airborne SV40 virus.

Considering the wide use of SV40 virus for oncogenic studies and the unusual airborne stability reported herein, it is obvious that laboratory procedures must be carefully assessed with respect to creating biohazardous aerosols. Moreover, because of their airborne stability, the inhalation route for oncogenic viruses must not be excluded as a means of accidental transmission.

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