Effect of Temperature and Relative Humidity on Survival of Airborne Columbia SK Group Viruses

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ABSTRACT

AKERS, T. G. (Naval Biological Laboratory, University of California, Berkeley, Oakland), SHEILA BOND, and L. J. GOLDBERG. Effect of temperature and relative humidity on survival of airborne Columbia SK group viruses. Appl. Microbiol. 14:361–364. 1966.—Three strains of the Columbia SK (Col-SK) group of viruses [Mengo, Maus Elberfeld (ME), and Col-SK viruses] have been studied in the airborne state. All three strains were found to give identical aerosol decay patterns at 16 or 26 C, when held at the same relative humidity (RH). During the first 5 min of aerosol storage time at 16 C, virus inactivation was RH-dependent, with survival maximal at either high (greater than 80%) or low (less than 5%) RH. After 5 min at 16 C, further inactivation, regardless of RH, was insignificant. At 26 C, the effect on survival of RH between 40 and 60% was even more pronounced than at 16 C, and continued after 5 min through 6 hr. Results of this study indicated that the inactivation of airborne Col-SK group viruses was similar to that of other ribonucleic acid (RNA) viruses, particularly poliovirus. Since members of the Col-SK group are picornaviruses, they may well serve as an aerosol model representative of small, ether-resistant, single-stranded RNA viruses.

The Mengo, Maus Elberfeld (ME), and Columbia SK (Col-SK) viruses are members of the Col-SK group of viruses. Although originally isolated from a variety of mammalian sources, individual strains are immunologically indistinguishable (7). Viruses in this group are classified as picornaviruses, are pathogenic for mice, and replicate rapidly in, and are easily assayed with, L cells. These characteristics make the Col-SK group viruses ideal models of the class of small, ether-resistant, single-stranded ribonucleic acid (RNA) viruses for use in aerosol studies.

Results of aerosol studies with the Col-SK group of virus strains are reported herein. These studies were designed to determine whether the three virus strains are identical in aerosol behavior, and whether their airborne characteristics are similar to those previously reported for poliovirus (4, 5).

MATERIALS AND METHODS

Viruses. Col-SK and Mengo viruses were obtained as 20% mouse brain suspensions from H. M. S. Watkins of the Naval Biological Laboratory. The ME virus, received as infective tissue culture fluid, was kindly supplied by R. Rueckert, University of California. The three strains were serially passaged 10 times in L-cell cultures before being used to prepare pools for the aerosol experiments.

Stock suspensions were prepared by inoculating 2 to 3 ml of virus onto confluent L-cell monolayers grown in 16-oz prescription bottles. After 30 to 45 min, excess inoculum was drained off, and 10 ml of Eagle's minimal essential medium (MEM) was added. Inoculated cell monolayers evinced advanced cytopathic effects (CPE) between the 9th and 12th hr. At this time, bottles were subjected to three sequential cycles of freezing and thawing, followed by pooling of their contents and subsequent storage at −70 C. Before aerosolization, the frozen virus suspension was thawed and then clarified by centrifugation at 3,000 × g for 5 min. Such virus suspensions uniformly titered 2 × 106 to 3 × 108 plaque-forming units (PFU) per ml.

Tissue culture. Mouse fibroblast (strain L-929) cells were obtained from the American Type Culture Collection. Stock cultures were grown in 16-oz glass prescription bottles, with MEM supplemented with antibiotics (100 units of penicillin per ml and 100 μg of streptomycin per ml) and 10% inactivated (30 min at 57 C) fetal calf serum. The pH was adjusted to 7.3 with...
sodium bicarbonate. Seventy-eight serial passages of this cell line have been completed in this laboratory, and routine testing has shown the cells to be free from pleuropneumonia-like organisms (PPLO).

Cells were removed from the glass surface of the bottles with saline-trypsin-Versene (STV) solution (6). After centrifugation to remove STV, followed by resuspension in MEM, a fully confluent monolayer yielded a total of 2.5 × 10⁶ cells per bottle.

To prepare plates for virus assay, 5-ml quantities of cell suspension (diluted to give 10⁴ cells per milliliter) were dispensed into plastic dishes (16 by 50 mm; Falcon Plastic, Div. of B-D Laboratories, Inc., Los Angeles, Calif.); these were incubated at 37°C in an atmosphere with 95% relative humidity (RH) consisting of 5% CO₂ in air. A confluent monolayer, suitable for inoculation, was obtained after 24 to 48 hr.

**Virus assay.** The plaque assay method of R. R. Reuckert (personal communication), with slight modifications, was employed in this study. The virus specimen to be assayed was serially diluted 10-fold in MEM without antibiotics or serum and inoculated in 0.2-ml amounts onto drained, unwashed plates, with three plates per dilution. Inoculated plates were returned to the CO₂ incubator (Hotpack, Philadelphia, Pa.) for 30 min at 37°C to allow adsorption to occur. Plates were then overlaid with a solution containing 1.5% Methocel (4,000 centipoises, Dow Chemical Co., Midland, Mich.), 0.2% glucose, 0.1% bovine serum albumin (BSA), Eagle's essential amino acids and vitamins, and Earle's salts. The overlay was dispensed in 8.0-ml amounts over confluent monolayers by means of an all-glass Aupet (Clay and Adams Co., New York, N.Y.). The use of Methocel overlay proved to be efficient. Unlike agar, it has a viscosity which is not sharply temperature-dependent, and, once prepared, it could be stored up to 1 week at 4°C and then dispensed after simply warming to room temperature.

After inoculation, adsorption, and overlaying, the plates were returned to the CO₂ incubator at 37°C to permit plaque development. Plaques were counted after 3 to 4 days of incubation.

The Methocel overlay was removed by aspiration. The remaining unwashed cell sheet was stained by carefully adding 5.0 ml of neutral red (1:10,000) solution to each plate, which was then placed in the dark for 30 to 60 min. The stain was removed by aspiration prior to counting the plaques.

**Aerosolization.** Virus aerosols were held in NBL rotating drums (3) according to the procedure of Watkins et al. (8). For this study, a holding temperature of 16 or 26°C and an RH range of 5 through 95% were used. At least two experiments (runs) were made for each RH selected at either 16 or 26°C, and every impinger sample was divided and assayed at least twice. A modified Wells' reflux atomizer was operated for 10 min, with a pressure of 10 psi, with the resultant aerosol entering a drum during this period of atomization. A fresh 10-ml sample of infected tissue culture fluid was used for atomization at each RH studied. Col-SK group virus titers before and after a 10-min refluxing period in the atomizer ranged from 2 × 10⁶ to 3 × 10⁶ PFU/ml. It was evident that refluxing of the suspension within the atomizer during the process of atomization was not deleterious to these viruses.

The Wells' atomizer aerosolized approximately 0.1 ml of fluid per min; hence, approximately 1.0 ml of virus material of an estimated titer of 10⁹ PFU/ml was delivered per drum. After the virus was aerosolized, the drum was allowed to equilibrate for 5 min prior to sampling. Uniform distribution of aerosol within the drum was aided by starting and stopping the drum rotation several times during this 5-min period.

Aerosol samples were taken after the 5-min mixing period and at hourly intervals during the next 6 hr, and were collected in an AGI-30 raised impinger (12.5 liters per min at critical flow rate) containing 20 ml of MEM fluid plus 0.1% antifoam (Dow Corning Antifoam B). Sampling time was 5 min. Virus content was measured by plaque assay and expressed as plaque-forming units per milliliter of impinger fluid.

**RESULTS**

**Decay rate and effect of frozen storage.** Impinger samples from a number of drum runs were divided into three samples. The first series was assayed immediately; the second and third series were stored at −70°C and assayed 1 week and 6 months later, respectively. Virus samples collected from aerosols were found to be stable when stored at −70°C, and impinger fluids could be assayed at any time after sampling. Results of assays of impinger samples collected from ME virus aerosols at two relative humidities are illustrated in Fig. 1. Similar findings were observed for all relative humidities, as well as for the two other virus strains studied. The apparent incongruity of the 5-min airborne concentration of virus at 38 and 54% RH (Fig. 1) is due to the
initial rapid virus inactivation which occurs during the first 5 min at 38 to 40% RH. As seen in Fig. 2, the 5-min virus aerosol concentration at 54% RH is nearly 2 logs greater than at 38% RH.

**Virus recovery.** Figure 2 summarizes the data on ME virus aerosol infectivity at 26 C. Virus survival was a function of RH and time. At relative humidities of 75% or more, ME virus aerosols retained high levels of infectivity during the 6-hr sampling period. However, at the mid-range of 40 to 60% RH, there was a rapid decline with time of airborne virus survival. For example, at 50% RH, a 5-log decrease in virus titer was observed in samples taken after 5 min and at 2 hr. In contrast, at 20 or 75% RH, the decrease in virus titer was observed to be less than 2 logs between samples collected at 5 min and at 6 hr.

The results of assays of Mengo, Col-SK, and ME virus aerosols after 6 hr at 26 C are shown in Fig. 3. No significant differences were observed with the respective survival patterns for the three strains. Similar survival curves were obtained for the three strains regardless of aerosol age or temperature.

The effect of RH on ME virus aerosols at 16 C is shown in Fig. 4. Similar results were obtained with Mengo and Col-SK viruses. By comparing Fig. 2 and 4, it is evident that the survival characteristics of Col-SK group viruses, although RH-dependent, were established within 5 min after the introduction of aerosolized virus into the rotating drums. The percentage recovery after 5 min was similar at 16 or 26 C, and survival patterns at both temperatures were characterized by greatest loss between 40 and 60% RH. The deleterious effect of mid-range relative humidities on airborne survival does not appear to be tem-
temperature-dependent. Because only two holding temperatures were studied, the effects of thermal inactivation on airborne viruses cannot be assessed in this study. However, it was observed that at 26°C there was detectable virus inactivation throughout the 6-hr study period, whereas at 16°C there was relatively little virus inactivation after the initial 5 min.

**DISCUSSION**

In comparing results of this study with other aerosol studies reported in the literature, one is confronted by the fact that only rarely have investigators working with viruses used comparable suspending media or techniques and systems. Procedural variations are such that some reports are based on aerosol ages measured in seconds, whereas other reports on aerosol aging are in terms of hours. To date, the limited number of viruses aerosolized under comparable conditions precludes conjecture regarding virion components and their structural relationship to airborne inactivation. Nevertheless, the aerosol studies at 26°C are interesting in that the inactivation curves for the Col-SK group viruses are similar to those observed with vesicular stomatitis (8), Rous sarcoma (9), measles virus (1), poliovirus (4, 5), and T-3 coliphage (2). These viruses, when airborne, are RH-dependent. Consistent recovery of virus is observed at low or very high RH and with rapid virus inactivation within the RH range of approximately 40 to 60%. Results of experiments reported herein at 16 and 26°C indicate the establishment of RH-dependent inactivation patterns which were evident within 5 min after aerosolization and persisted through 6 hr. This observation supports the validity of assaying after short time periods, as reported by Harper (4), who studied aerosols 1 sec after spraying, and by Hemmes et al. (5), who initiated their studies after 30 sec.

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**LITERATURE CITED**