Virucidal Properties of Light and SO_2
II. Effect of a Low Gas Concentration on Aerosolized Virus (36038)

RICHARD F. BERENDT, EMERSON L. DORSEY, AND HENRY J. HEARN
Aerobiology and Evaluation Laboratories, Fort Detrick, Frederick, Maryland 21701

In a previous study, we investigated the virucidal properties of SO_2 in the presence of 40 and 308 mcal cm^{-2} min^{-1} of simulated solar radiation, using the virus of Venezuelan equine encephalomyelitis (VEE) as a model for test. A concentration of 3.6 ppm SO_2 was arbitrarily selected as a test condition because it represented a value that was considered to be close to the maximal allowable concentration for a human environment (1). Results of the tests, which were performed at 30 and 60% relative humidity (RH) showed that both the radiation and the pollutant alone produced adverse effects on the aerosolized virus. When the 308 mcal cm^{-2} min^{-1} light intensity was combined with the pollutant, viral decay rates at both relative humidities were significantly greater than the sum of the separate effects. At the 40 mcal cm^{-2} min^{-1} level of light intensity, a similar effect was observed at 60% RH, although the magnitude of viral inactivation was not as great as when the higher light intensity was used. At 30% RH, radiation and SO_2 also showed an effect on the aerosolized virus, but, in contrast to results above, the inactivation rate was significantly less with the combination than with the pollutant alone. The combined inactivation rate was not significantly lower, however, than that of the light alone.

Thus, data indicated that the virucidal activities of light and SO_2 were increased when they were used together, but that under certain environmental conditions, the viral inactivation by SO_2 appeared to be partially negated by the light.

In an effort to define more completely the virucidal consequences of the interaction of simulated solar radiation and SO_2, a series of experiments was conducted in which the pollutant was employed at different concentrations. Results presented below were obtained with a gas concentration that was approximately one-tenth that used in previous experiments (3).

Methods and Materials. Virus. The suspension of Trinidad strain of VEE virus was prepared after 10 passages in chick fibroblast monolayers. The virus was harvested in 0.02 *M* phosphate buffer, pH 7.5, after passage 10 and then was stabilized by the addition of 0.3% bovine serum albumin (BSA). The preparation was centrifuged at 1000 rpm for 10 min to remove cellular debris and then at 20,000 rpm for 2.5 hr to sediment the virus particles. The centrifuged material was resuspended in 0.02 *M* phosphate buffer, pH 7.5, containing 0.3% BSA, dispensed in 2-ml portions, and frozen at —60° until used. These preparations contained about $10^{9.3}$ plaque-forming units (PFU)/ml.

VEE virus assay. Aerosol samples of VEE virus were collected and diluted in heart infusion broth, and the viable virus concentration was assayed by plaque formation on chick fibroblast monolayers. Five milliliters of chick fibroblasts (8×10^6 to 10×10^6/ml) suspended in growth medium (Eagle's basal medium, 500 ml; fetal calf serum, 50 ml; lactalbumin hydrolysate, 25 ml; plus penicillin, streptomycin, and Aureomycin) was seeded onto plates and incubated overnight in a 5% CO_2 atmosphere at 35°. Prior to virus assay, the liquid was decanted and the plates were then inoculated with 0.1 ml of the appropriate virus dilution and allowed 30 min for viral attachment. Five milliliters of an agar overlay (growth medium plus 1% Noble agar) was added to each plate before incubation in a 5% CO_2 atmosphere for 36 hr at 35°. One milliliter of 0.1% neutral red was placed directly on top of the overlay, and

the excess was decanted after a few minutes. Plates were incubated for 6 additional hr, the plaques were counted, and the concentrations were recorded (PFU/ml of sample).

Aerosol dissemination and sampling. Aerosols of virus were disseminated with a FK-8 gun into a 650-liter revolving drum, equipped with a simulated solar radiation source as previously described (2). The technique employed for sampling with AGI impingers and assay of virus have also been described previously (3).

Irradiation procedures. As in previously reported work (3), two levels of solar radiation were simulated with a xenon source: 308 and 40 mcal cm^{-2} min^{-1}. The energies found in the 300 to 400 nm band for each of these total intensities were 8 and 2 mcal cm^{-2} min^{-1}, respectively.

Pollutant generation and assay. The concentration of gas employed in these experiments was 0.4 ppm. The SO_2[1] was introduced into the airstream that was employed to attain the desired relative humidity level in the drum, as described previously (3). Briefly, the drum was flushed with air containing SO_2 for 10 min; the airflow was stopped; and, after an additional 10-min period, the virus was generated. Pollutant concentrations were determined by the peroxide method described by Jacobs and Greenburg (4) and Hochheiser (5). At no time did the SO_2 concentration in the background air reach detectable limits.

Calculations. Percentage recovery values were calculated as the ratio of the concentration of virus recovered at a given time to that originally disseminated. Decay rates were calculated by a least squares fit to the exponential model:

$$c_t = c_0 e^{-kt}$$

where c_t is the percentage recovery at cloud age (min), c_0 is an estimate of the percentage recovery at zero cloud age, and k is the regression coefficient of aerosol concentration on cloud age (this value is usually expressed as $100k = \%/\text{min}$).

Four replications of each treatment were carried out in a completely randomized experiment.

Results. Plots of the loss of viability of the virus as a function of time under various conditions of treatment at 30 and 60% RH are shown in Figs. 1 and 2, respectively. Decay of the virus in a dark atmosphere containing 0.4 ppm of SO_2 was significantly greater than that in the absence of the pollutant at both RH levels. Perhaps the most striking observation, however, was the apparent inhibition of the virucidal effects of light by the presence of 0.4 ppm of SO_2. This effect was most marked at 60% RH with the 308 mcal cm^{-2} min^{-1} intensity. In this case, the decay rate of the virus decreased from 14.3%/min in the presence of the light alone to 9.1%/min when 0.4 ppm of SO_2 was introduced into the atmosphere prior to dissemination of the virus suspension. The ameliorative effect of the pollutant was also noted with the other conditions of relative humidity and light in-

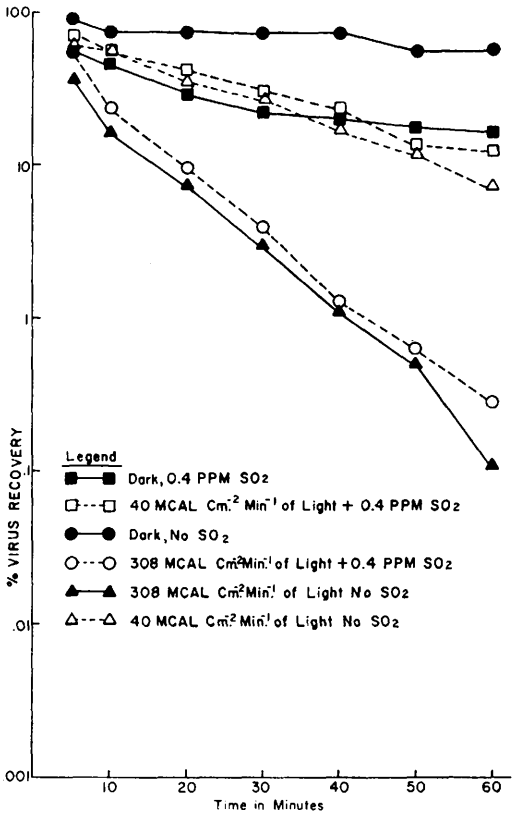

FIG. 1. Reaction of VEE virus to SO_2 and simulated solar radiation at 30% RH.

[1] SO_2, 1000 ppm; Matheson Gas Products, East Rutherford, NJ.

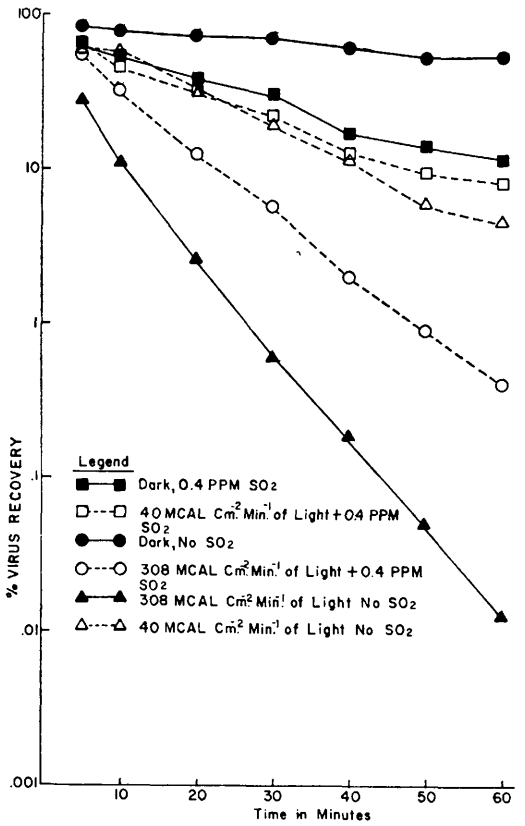

FIG. 2. Reaction of VEE virus to SO_2 and simulated solar radiation at 60% RH.

tensity, but the magnitude was not as great.

Discussion. In a previous report (3), we presented data showing that 3.6 ppm SO_2 and 308 mcal cm^{-2} min^{-1} of light were more virucidal for airborne VEE virus, when used in combination, than would be expected from the sum of their individual effects at relative humidities of 30 and 60%. When 40 instead of 308 mcal cm^{-2} min^{-1} were used, a less intense but similar effect occurred at 60% RH. At 30% RH, however, viral inactivation was significantly lower with the combination than with the SO_2 alone. The fact that the combination remained more effective than light alone but became less effective than SO_2 alone suggested that, under certain circumstances, light was capable of reducing the virucidal properties of SO_2. This concept was supported by additional data showing that when SO_2 was generated into the aerosol chamber and treated with light and then the light was switched off prior to aerosolization of the virus, the virucidal property of the gas was diminished.

Results obtained in the present experiments with the same light intensities (308 and 40 mcal cm^{-2} min^{-1}) but with 0.4 ppm SO_2 differ from those obtained previously with 3.6 ppm SO_2 in several important respects: (i) In the present experiments, 308 mcal cm^{-2} min^{-1} of light alone were significantly more virucidal than the light–SO_2 combination at 60% RH; the combination remained considerably more effective, however, than SO_2 alone. Thus, it appears in this case that the SO_2 interfered with the virucidal properties of the light in contrast to previous results, which suggested that the light interfered with SO_2. (ii) Only slight differences in viral inactivation were found with 40 mcal cm^{-2} min^{-1} of light and SO_2, and with light alone. Thus, while virus appeared to be inactivated to some extent by the 0.4 ppm SO_2 and 40 mcal cm^{-2} min^{-1} of light, no evidence of a synergistic effect of both factors was found. It is possible that, under these circumstances, the light and SO_2 may have been antagonistic to one another, thereby reducing a possible enhanced combined effect. The data provide some evidence of this because, as shown in Figs. 1 and 2, viral decay rates were always slightly less with the combination than with light alone. (iii) The most obvious ameliorative effect of 40 mcal cm^{-2} min^{-1} of light on the virucidal properties of 3.6 ppm of SO_2 in the previous study was found at 30% RH, whereas, in the present work, the ameliorative effect of the 0.4 ppm of SO_2 on 308 mcal cm^{-2} min^{-1} occurred at 60% RH.

The data obtained thus far, however, are not sufficient to discern the mechanism(s) of action of light or SO_2 in the phenomena described above. The negation of one factor by the other may have resulted because some wavelengths of solar radiation were absorbed by SO_2 or because SO_2 may have reacted with sunlight, leading ultimately to the production of sulfuric acid, as discussed by Leighton previously (6). A theory based upon a mutual antagonism between the light and SO_2 activity does not take into account the possibility

that a viral protective substance may be formed and, if so, whether it acts at a site on the virion itself. Moreover, the fact that, under certain circumstances, the combined light–SO_2 action was more virucidal than the sum of each effect strongly suggests that a variety of reactions are capable of taking place. Further studies to elucidate these phenomena are in progress.

Summary. A concentration of 0.4 ppm of SO_2 in air is toxic for airborne Venezuelan equine encephalomyelitis virus. When the SO_2 is irradiated by either 40 or 308 mcal cm^{-2} min^{-1} of simulated solar radiation, the gas appears to interfere with the virucidal property of the light. This reaction is most marked with the 308 mcal cm^{-2} min^{-1} level at 60% relative humidity, but it also occurs to a lesser extent with the 40 mcal cm^{-2} min^{-1} intensity at both 30 and 60% RH.

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