Virucidal Properties of Light and SO_2
I. Effect on Aerosolized Venezuelan Equine Encephalomyelitis Virus (36063)

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Recent scientific literature contains many reports on the effect of air pollutants upon the susceptibility of various hosts to a number of infectious or neoplastic diseases (1–3). Very little information, however, has been published on the reaction between pollutants and airborne infectious agents exposed to sunlight. We have recently reported that simulated solar radiation had an adverse effect upon the survival of Venezuelan equine encephalomyelitis (VEE) virus as manifested by its loss of infectivity for chick fibroblast cell cultures (4). It was the purpose of this study to examine this phenomenon in greater detail by studying the effect of simulated solar radiation and SO_2, a common air pollutant, on virus survival. Because of the abundance of background information and the desirable characteristics of VEE virus for such a study, it was used as the model organism for this work.

Methods and Materials. Aerosol dissemination and sampling. Aerosols of virus were disseminated with a FK-8 gun into a 650-liter revolving drum that was equipped with a simulated solar radiation source. The equipment and dissemination techniques have been described in a previous publication (5). Samples were obtained for each experimental condition by passing the aerosol through AGI impingers (sampling rate: 6 liters/min) which contained 25 ml of heart infusion broth (Difco). Diluted aliquots of the heart infusion broth were assayed for virus by the plaque technique on chick fibroblast cells. Further description of the AGI impinger and its use in aerosol sampling can be found elsewhere (4). Samples of airborne virus were obtained at selected intervals over a 1-hr period.

Irradiation procedures. Solar radiation was simulated with a xenon light as previously described (5). Two light intensities, 40 and 308 mcal cm^{-2} min^{-1}, were employed (wavelength range was 300 to 2500 nm). Energy in the near-ultraviolet range (300 to 400 nm) for each of these intensities was 2.0 and 8.3 mcal cm^{-2} min^{-1}, respectively.

The 40 and 308 mcal cm^{-2} min^{-1} intensities employed are equivalent to the natural radiation measured about 30 and 60 min, respectively, after sunrise in early April at a latitude of 39°27'N.

Pollutant generation and assay. The SO_2[1] was introduced into the airstream used to attain the desired relative humidity in the drum. The air had previously been passed through a variety of filters to reduce background pollution, and subsequent assays showed that the background never exceeded 0.1 ppm of acid-forming gases. The SO_2 concentration was determined by the peroxide method described by Jacobs and Greenburg (6) and Hochheiser (7). The entire system was flushed for 10 min at a rate of about 175 liters/min with humidity-conditioned air containing 3.6 ppm of gas. The airflow was stopped and after a 10-min period for equilibration, assays for SO_2 concentration were performed at 2, 4, 8, 16, 32, and 64 min. SO_2 assays were replicated 6 times for each experimental condition (*e.g.*, light, relative humidity) to establish the precision of the gas dissemination procedure prior to introducing the virus into the system. Although we noted a consistent tendency for the gas concentration to decrease slightly with time the loss was

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

TABLE I. Survival of Airborne VEE Virus Exposed to Sulfur Dioxide and Simulated Solar Radiation.

Relative humidity (%)	SO_2 conc (ppm)	Light intensity (mcal cm^{-2} min^{-1})	% Recovery[a] at 5 min	Decay rate[a] 5–60 min (%/min)
30	0	0	89.4 (15)[b]	0.79 (40)[b]
	3.6	0	12.3 (45)	8.46 (11)
	0	308	37.7 (6)	10.09 (17)
	3.6	308	5.0 (61)	24.96 (18)
60	0	0	94.1 (26)	1.29 (58)
	3.6	0	27.1 (24)	5.30 (23)
	0	308	28.0 (37)	14.33 (14)
	3.6	308	4.8 (40)	40.60 (16)

[a] Means of four determinations for each treatment. The values for irradiated virus in the presence of SO_2 were derived for shorter periods than 60 min.

[b] Values in parentheses are coefficients of variation as per cent.

not statistically significant.

Calculations and experimental design. Percentage recovery values were calculated as the ratio of the concentration of virus recovered at a given time to the concentration 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 t (min), c_o is an estimate of the percentage recovery at zero cloud age, and k is the regression coefficient of aerosol concentration on cloud age (usually expressed as 100 k = %/min).

The experiment was completely randomized, with four replications of each treatment. For this study, statistical analysis was confined to t tests to determine the significance of the differences between means.

Results. In an effort to determine test conditions that produced an effect but allowed sufficient virus survival for adequate study, preliminary experiments were carried out with several concentrations of gas and selected light intensities. As examples of results obtained from such experiments, a combination of 10 ppm of SO_2 and 584 mcal cm^{-2} min^{-1} of simulated solar radiation lowered the recovery of VEE virus from aerosols below detectable limits in less than 20 min. In contrast, 3.6 ppm ($\pm 21\%$)[2] of pollutant and a light intensity of 308 mcal cm^{-2} min^{-1} permitted recovery of viable airborne virus for as long as 40 min. This concentration of SO_2, which is slightly less than that (5 ppm or greater) considered dangerous by the Los Angeles County Air Pollution Control District (8), and light intensities of 308 and 40 mcal cm^{-2} min^{-1} were used as test conditions for further study.

Initially, four replicate experiments of each treatment were carried out at each of two relative humidity (RH) levels, 30 and 60%. The environments investigated included: nonirradiated pollutant-free (control), nonirradiated in the presence of 3.6 ppm of SO_2 308 mcal cm^{-2} min^{-1} of simulated solar radiation in the absence of SO_2, and a combination of SO_2 and irradiation. Later investigation was carried out with 40 mcal cm^{-2} min^{-1}. Determinations with the 12-channel radiation sensors (5) showed that the intensity of the shortest wavelength range of radiation (300 to 400 nm) was 8.3 mcal cm^{-2} min^{-1}.

Effect of SO_2 and irradiation. The results from the trials at 30 and 60% RH are given in Table I and are plotted in Figs. 1 and 2. VEE virus survived in aerosols with no significant decay when radiation and the pollutant were not present. In the presence of 3.6 ppm of SO_2, the decay rate of aerosolized virus ranged from 5 to 8%/min and was

[2] Coefficient of variation of the mean of 18 determinations.

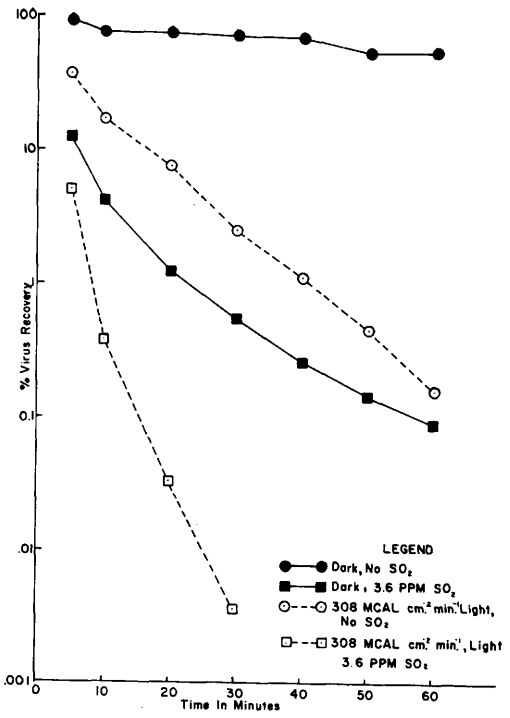

FIG. 1. Effect of SO_2 and simulated solar radiation (308 mcal cm^{-2} min^{-1}) on airborne VEE virus at 30% RH.

significantly greater than that of the non-SO_2 control ($p \leq .01$). In the presence of light without pollutant the virus also decayed very rapidly, confirming our previously reported observation (4). When aerosolized virus was both irradiated and treated with SO_2, the decay rate of the virus was very high at both RH levels (from 24 to 41%/min) and differed from the control decay rate at a high level of significance ($p \leq .001$).

Effect of relative humidity. In contrast to results obtained with untreated aerosolized virus, relative humidity appeared to exert an effect on the virus in the presence of SO_2 and 308 mcal cm^{-2} min^{-1} of light. As shown in Table I, the adverse effect of SO_2 on the virus at 30% RH was significantly ($p \leq .01$) more pronounced than that at 60%. At 60% RH, the effect of radiation in a pollutant-free atmosphere was significantly greater ($p \leq .05$) than at 30% RH (decay rates of 14.3 and 10.1%/min, respectively). At both humidities, the effect of SO_2 and simulated solar radiation in combination was greater than the sum of their separate effects, suggesting that an interaction had taken place. This combined effect was significantly greater, however, at 60% RH than at 30% ($p \leq .01$).

Effect of low light intensity. In an effort to confirm and extend results suggesting an interaction between SO_2 and radiation, experiments were repeated with a reduced light intensity (ca. 40 mcal cm^{-2} min^{-1}). The data obtained from 4 replicate trials at 30 and 60% RH are given in Figs. 3 and 4. The dark control data from the previous trials are shown for comparison. As shown, this low level of irradiation inhibited viral activity, but not to the extent found with the high light level (Figs. 1 and 2). In contrast to previous findings, however, the combination of low irradiation and 3.6 ppm of SO_2 was significantly less toxic for the virus than was the gas alone at 30% RH ($p \leq .001$). This unique result was not found at 60% RH; in this case, as with high light intensity, the combination of gas and low light again produced a greater adverse effect upon the virus than the sum of those occurring after sepa-

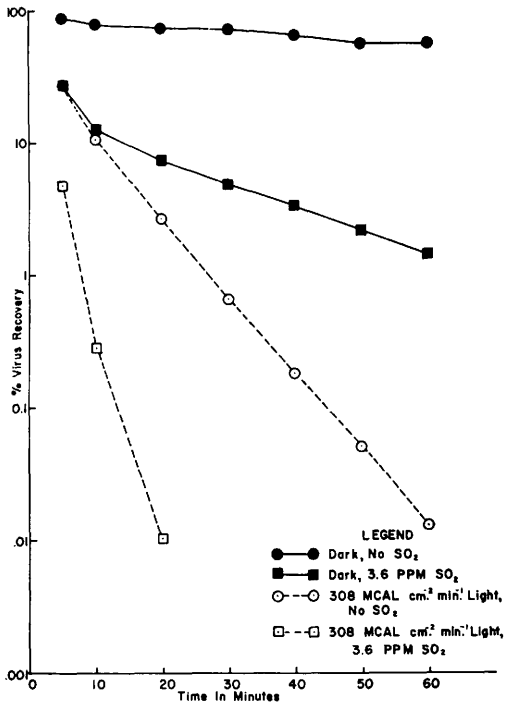

FIG. 2. Effect of SO_2 and simulated solar radiation (308 mcal cm^{-2} min^{-1}) on airborne VEE virus at 60% RH.

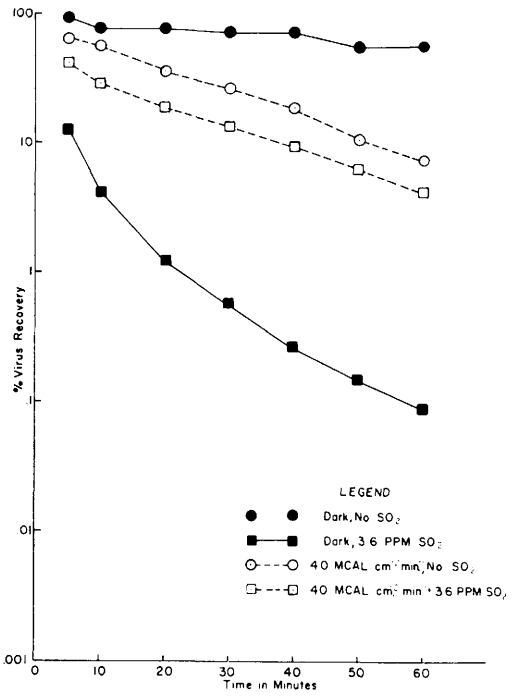

FIG. 3. Effect of SO_2 and simulated solar radiation (40 mcal cm^{-2} min^{-1}) on airborne VEE virus at 30% RH.

rate treatments.

Test conditions in the foregoing experiment required aerosolized virus to be present with light and/or SO_2. Inasmuch as low light intensity at 30% RH had an ameliorative effect on the virucidal properties of SO_2, an additional series of tests was performed to determine whether light exerted its modifying effect directly on SO_2. Data obtained in these tests revealed that when 3.6 ppm of SO_2 were treated with 40 mcal cm^{-2} min^{-1} of light prior to aerosolizing the virus, viral recoveries again were higher than with SO_2 alone. These data were interpreted as suggesting that the low light intensity reduced the virucidal properties of the gas at 30% RH, thus favoring viral survival.

Discussion. We have shown that SO_2 (3.6 ppm) and simulated solar radiation at a level of 308 mcal cm^{-2} min^{-1} are highly deleterious to the survival of aerosols of VEE virus. The data indicate, however, that this effect can be altered at different relative humidities. As one example, SO_2 was more effective without light against the virus at 30% RH than at 60% RH, despite reports that when SO_2 is used as a germicide, a minimum 60% RH level is required (9, 10). Of greater complexity, however, were data showing that, when the light intensity was 308 mcal cm^{-2} min^{-1}, the data at 30% RH seemed to indicate a different kind of interaction, since the combination of light and SO_2 was less toxic for the virus than the SO_2 alone. Taking our data into account, possibly, SO_2 fumigations that take place in the presence of light may require a high humidity to ensure an adequate effect.

Thus far, the data provide few clues to the mechanism(s) of viral inactivation that occurred in the present study. The end product of irradiation of SO_2 in air is an aerosol of sulfuric acid (11, 12) that could be deleterious to VEE virus. If this is the case, the question of the role that light may play in augmenting the viral inactivation remains unanswered, as does the influence of environ-

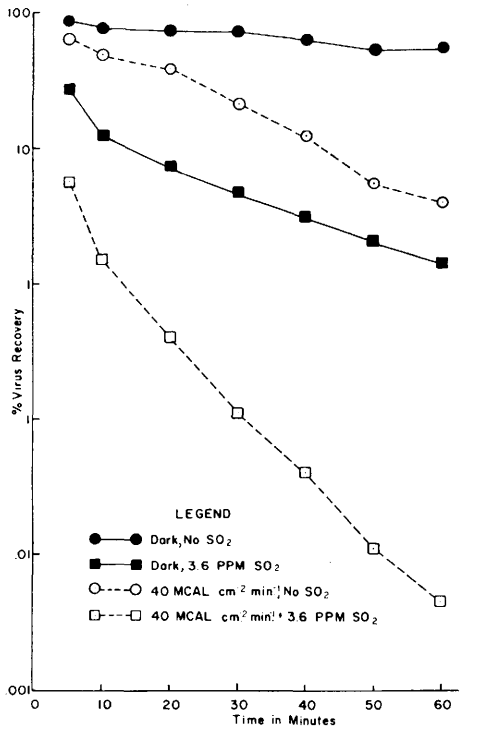

FIG. 4. Effect of SO_2 and simulated solar radiation (40 mcal cm^{-2} min^{-1}) on airborne VEE virus at 60% RH.

mental humidity. It is unlikely, however that the conversion of SO_2 to H_2SO_4 is important here, since the rate of conversion is usually less than 1%/hr (12, 13).

Light by itself in the range of 330 to 470 nm can be virucidal, as evidenced by the report of Appleyard (14), who worked with Semliki Forest virus suspension. In our studies with aerosolized virus using the highest light intensities (308 mcal cm^{-2} min^{-1}) at 30 and 60% RH, presumably only small amounts of energy that were within the 300 to 400 nm range of the spectrum entered the system, but this still might account for the light inactivation that was found. With the lowest intensity (40 mcal cm^{-2} mn^{-1}), which provided 2 fcal cm^{-2} min^{-1} within the 300 to 400 nm range, a concomitant decrease in viral inactivation occurred at both humidities. Nevertheless, at 60% RH, the light–SO_2 combination was still most highly effective in inactivating virus. In marked contrast, at 30% RH, this same combination was only slightly more inactivating than the light and considerably less effective than the SO_2 alone. Our data suggest that at 30% RH, the low intensity of light acted upon the SO_2 directly, preventing it from attacking the virus.

Obviously, a great deal more research is necessary to elucidate the phenomena and to determine whether other viruses, particularly those that are respiratory, are similarly affected. These studies will be the subjects of further communications.

Summary. A combination of 3.6 ppm of SO_2 gas and 308 mcal cm^{-2} min^{-1} of simulated solar radiation were highly toxic in tests employing airborne Venezuelan equine encephalomyelitis virus as a model. Under these conditions, the virus decayed more rapidly at 60% RH than at 30% RH (decay rates of 40%/min vs 25%/min, respectively). Although both pollutant and light were toxic when employed separately, the viral response to a combination of the two was much greater than the sum of the separate effects, suggesting that an interaction had occurred. Lowering the light intensity to 40 mcal cm^{-2} min^{-1} produced a similar although decreased effect at 60% RH. At 30% RH, however, the SO_2–light combined effect was not found; in this case, SO_2 alone effected viral inactivation to a significantly greater degree than either light alone or the SO_2–light combination. The data suggest that, in this case, the low intensity of light acted upon the SO_2 directly, preventing it from attacking the virus.

The authors thank Charles Veirtz, Leonard Wilhelm, Richard DeLauter, and Donald Phebus for technical assistance.

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Received Feb. 22, 1971. P.S.E.B.M., 1972, Vol. 139.