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# Effect of Relative Humidity on the Inactivation of Airborne *Serratia marcescens* by Ultraviolet Radiation

R. L. RILEY AND J. E. KAUFMAN

*Department of Environmental Medicine, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205*

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Apparatus was designed and constructed in which a bacterial aerosol of known age, particle size, and relative humidity (RH) could be exposed to ultraviolet (UV) radiation of measured intensity for a given period of time and then be sampled quantitatively. Aerosols of *Serratia marcescens* were exposed to UV dosages between 96.0 and 0.75 ($\mu$w-sec)/cm$^2$ at humidities ranging from 25 to 90%. A sharp decline in the fraction of organisms killed was found at RH values above 60 to 70%. Above 80% RH, there was evidence for reactivation induced by UV. The plot of "log fraction organisms remaining" versus UV dose was curvilinear, suggesting noncompliance with the monomolecular law of reaction velocity, but the Bunsen-Roscoe law of reciprocity between time and intensity of UV exposure was demonstrated to hold. These results could be accounted for by postulating the presence in the aerosol of two populations of organisms with different sensitivities to UV, each individually obeying the monomolecular law of reaction velocity. The data amplify existing information on the relationship between UV disinfection of airborne organisms and RH. In the middle range of humidities, the sensitivity of the organisms to UV was greater than would be expected from published reports.

In exploring the application of ultraviolet (UV) air disinfection in rooms for the purpose of reducing airborne infection (15-18), it became apparent that more complete information is needed on the relationship between relative humidity (RH) and the effectiveness of UV as an air disinfectant. Although a number of workers have reported decreased killing of airborne organisms by UV at humidities in excess of 60 to 70% (5, 10, 21, 22), there are statements in the literature denying any RH effect (12-14). This controversy needs to be resolved both as a contribution to basic knowledge of the germicidal effects of UV and as a basis for predicting the effectiveness of UV installations under various conditions of RH.

There is also need for more quantitative information regarding the killing power of UV for airborne organisms. In past studies, there have been difficulties related to the measurement of UV intensity, the variation in intensity at different distances from the tube, and the movement of airborne organisms in relation to the tube. We, therefore, undertook to design and construct an apparatus which obviated many technical difficulties and then to repeat studies of UV killing power for airborne *Serratia marcescens* at different humidities under controlled experimental conditions.

## MATERIALS AND METHODS

**UV exposure unit.** Exposure of the airborne bacteria to a given intensity of UV was carried out by passing the aerosol through a thin slot at a distance of either 4 or 8 feet from the UV source [Westinghouse Slimline Germicidal Lamp G10T5 with adjustable current flow (Fig. 1)]. A pane of polished Vycor glass was used for the roof of the exposure slot because of the UV transmitting qualities of this material. Part of the floor of the slot was removable so the intensity of UV could be measured directly with an accurate meter (3). Flow through the slot was standardized at 1 ft$^3$/min and was motivated by a Sears Kenmore vacuum cleaner pump at the downstream end of the apparatus (Fig. 2). Since the slot was 1 by 1 by 0.1 ft, all organisms were exposed to virtually the same intensity of radiation during the 0.1 min required to pass through the slot. For certain studies, arrangements were made for shorter exposures as described below.

The exposure unit was constructed of 0.25-inch (0.635 cm) Plexiglas. The aerosol and diluent air were first mixed in the region containing baffles (Fig. 1). The diluted aerosol then passed through a bank of parallel glass tubes to produce stream lines of flow of equal velocity. Experiments with smoke convinced us that the aerosol did in fact pass through the UV exposure slot in a smooth manner. The slot extended for 2 ft downstream from the UV exposure area to serve as a pressure-equalizing plenum. The flow was even across the entire cross section of the exposure slot and showed no visually detectable tendency to be faster toward the center.

It seemed important to eliminate reflected UV from the exposure slot so that all radiation impinging on the aerosolized organisms would be from above and hence measurable with our unidirectional UV meter. The tower holding the UV tube and the exposure slot were therefore painted black and coated with a black flock (Suede-Tex, Donjer Products Co.). The Vycor window was left uncoated.

**Aerosol production and control.** The organisms were atomized by a Wright nebulizer submerged in an ice bath and operated by compressed air at 15 psi. The airflow through the nebulizer was approximately 0.33 $ft^3$/min. The suspension or organisms in the reservoir of the nebulizer was made up by placing a frozen pellet of *S. marcescens* in 35 ml of 2% inositol and then pipetting 0.002 ml of this suspension into 25 ml of 2% inositol (19). It was found by trial and error that this dilution gave appropriate counts on the agar plates. The more concentrated slurry was kept in a refrigerator when not in use and was discarded after 3 days because experience indicated that counts began to fall after this length of time.

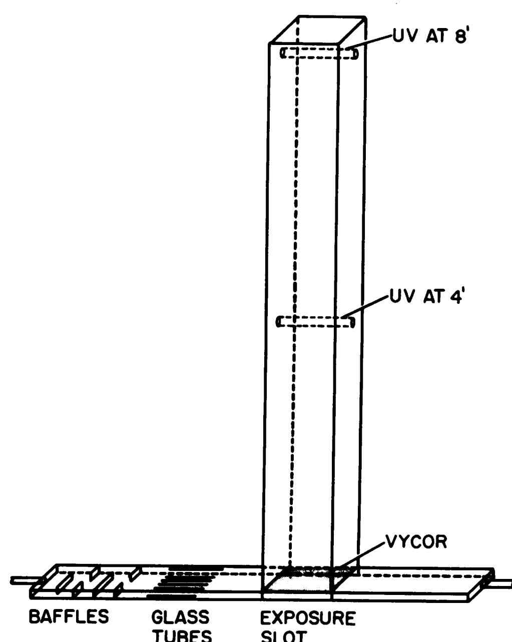


FIG. 1. *Apparatus for exposure of a bacterial aerosol to UV of known intensity.*

Diluent air was made up in different ways depending on the desired humidity of the mixed aerosol. Either room air, humidified air, or dry air was added to the aerosol from the Wright nebulizer before the mixture entered the UV exposure unit (Fig. 2). Mixtures with high humidity were constituted by drawing part of the diluent air from an open container full of mist produced by a Babbington nebulizer (9).

Downstream from the exposure unit, the aerosol moved in succession past dry and wet bulb thermometers for monitoring RH and then through a calibrated flow meter (F and P Co., precision bore flowrator tube 8427-10/27) to assure a flow of 1 $ft^3$/min at all times. A by-pass circuit permitted flow to continue at this rate in the intervals when samples were not being taken. Finally, the aerosol passed through the pump and was discharged into the exhaust system of the building.

**Aerosol sampling and counting.** A six-stage Andersen sampler was used to assess the viable organisms per cubic foot of aerosol and to determine the particle size distribution of the airborne particles (1). The entire flow of aerosol passed through the sampler when samples were being taken. The organisms were impinged on the surface of Trypticase soy agar, and the petri plates were incubated at 30 C for 24 hr. After the red colonies of *S. marcescens* were counted, a correction was applied, as recommended by the manufacturer, to take account of multiple hits. For each stage of the Andersen sampler, the ratio of the corrected colony count with UV on to the corrected count with UV off gave the fraction of organisms remaining after the given UV exposure. This fraction, when subtracted from 1.00, gave the fraction of organisms killed.

**Procedure.** Standard operation involved first turning on the UV, removing part of the floor of the exposure slot to take a direct reading of UV intensity in the slot, and adjusting the UV intensity to a predetermined level. The floor of the exposure slot was then replaced and made airtight with adhesive tape. The UV was turned off and the pump and the Wright nebulizer were started. Airflow was adjusted to 1 $ft^3$/min through both the sampling and bypass circuits. With flow through the bypass, the Andersen sampler was loaded with fresh agar plates.

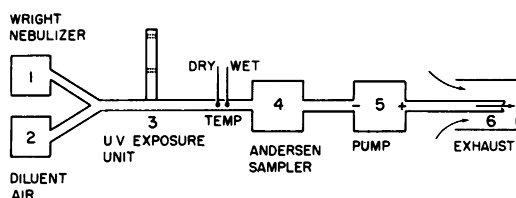


FIG. 2. *Block diagram showing train of apparatus for producing a bacterial aerosol, exposing it for a known period of time to UV of known intensity, characterizing it with respect to RH and particle size, and disposing of it to the outdoors.*

When flow had continued for at least 5 min to provide time for the aerosol to stabilize with respect to temperature and humidity, the UV was turned on for at least 3 min more for stabilization. The first "UV on" samples were then taken by redirecting the flow of aerosol from the bypass circuit to the Andersen sampler circuit. After exactly 1 min, sampling was terminated by switching back to the bypass circuit. The UV was then turned off and the Andersen sampler plates were changed. After a minimum of 3 min to restore equilibrium in the system with UV off, a set of "UV off" samples was taken. The UV was then turned on again, the humidity was set at a new level, and the procedure was repeated, always waiting a minimum of 3 min after any change for equilibrium to be reestablished. In this way, pairs of "UV on" and "UV off" samples were taken systematically at many different humidities and UV intensities.

After analysis of the results obtained with this protocol, we undertook studies in which UV exposure time was reduced. This was accomplished without altering flow by reducing the effective length of the exposure slot. For example, if we wished to reduce exposure time by half, a piece of cardboard was placed on top of the Vycor window to block UV from the downstream half of the slot. This reduced the effective length of the slot to 6 inches and reduced exposure time from 0.1 min (6 sec) to 0.05 min (3 sec).

## RESULTS

**Particle size distribution.** With UV off, the corrected colony counts for the various stages of the Andersen sampler provided information regarding the distribution of particle sizes in the aerosols (Table 1). This distribution is based on all counts with UV off over the entire range of RH, made during studies at 16, 3.2, and 1.7 μw per $cm^2$. Because most of the organisms were collected in stages five and six, the total corrected counts from these stages were combined in the analyses which follow, and counts from the first four stages were discarded. The fraction of particles in stage five increased relative to stage six as RH increased, indicating the expected increase in particle size with increase in RH (11).

TABLE 1. *Fraction of total counts in the six size ranges corresponding to the six stages of the Andersen sampler*

| Stage | Size range (μm) | Fraction of total |
|---|---|---|
| 1 | >9.2 | 0.009 |
| 2 | 5.5-9.2 | 0.01 |
| 3 | 3.3-5.5 | 0.015 |
| 4 | 2.0-3.3 | 0.071 |
| 5 | 1.0-2.0 | 0.562 |
| 6 | <1.0 | 0.332 |

**Effect of age of aerosol.** Since the airborne organisms were exposed to UV within about 12 to 15 sec of the time the aerosol was created, the possibility existed that the organisms were in an exceptionally fragile state. To compare their sensitivity to inactivation by UV to that of organisms held in the airborne state for a longer time, experiments were performed in which the aerosol was held for 5, 10, and 15 min in a large meteorological balloon. There was no indication that the organisms changed their sensitivity to UV over a 15-min time span.

**Percentage of organisms killed versus RH, at various doses of UV.** Almost all of the data in the present study can be summarized in a single graph where fraction of organisms killed (and fraction remaining) is plotted against RH (Fig. 3). The UV dosage (intensity × time of exposure) is shown for each of the curves. The data from which the curves in Fig. 3 were drawn are shown in Fig. 4 and 5.

At all dosages, a sharp decrease in fraction of organisms killed at high humidities was observed. Evidence of reactivation of organisms (negative fraction killed) was seen at very high humidities.

The curves in Fig. 3 become increasingly

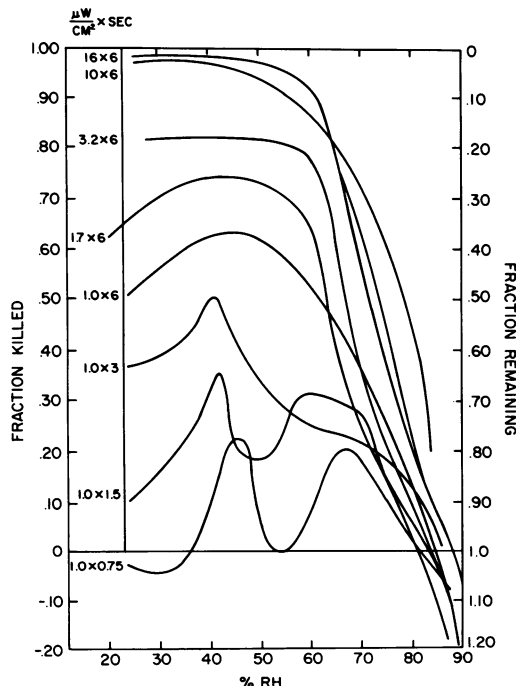


FIG. 3. *Graph of fraction of organisms killed (and fraction remaining) versus RH at eight different doses of UV [intensity: (μw/cm²) × time in sec].*

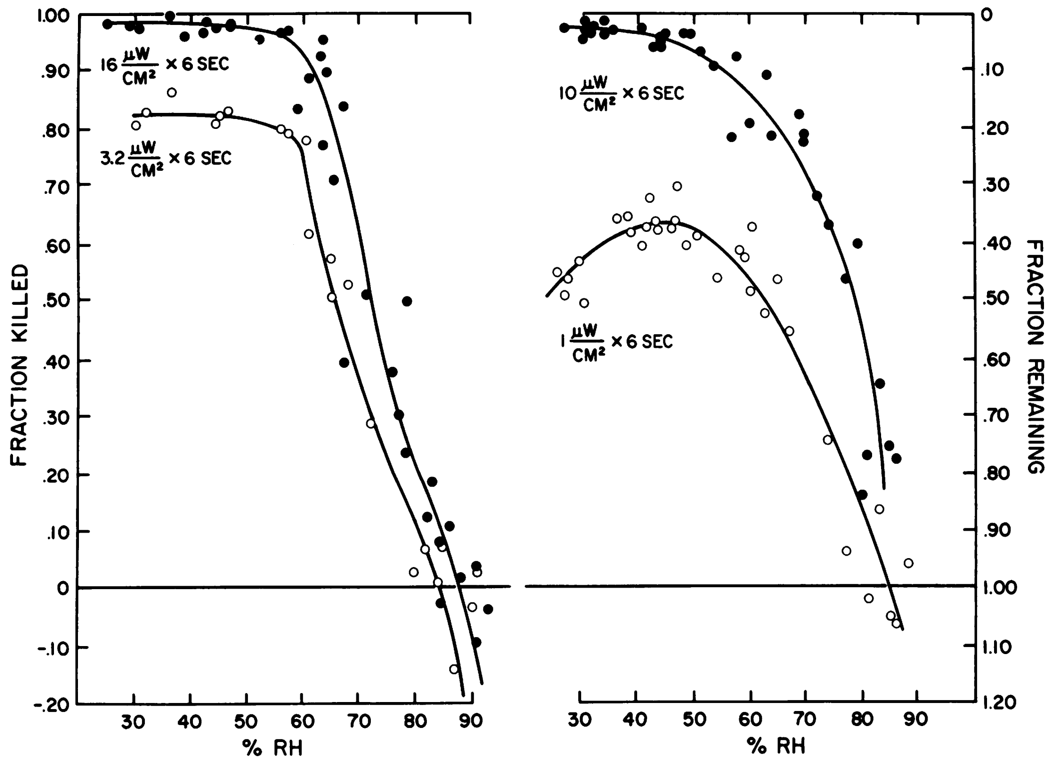


FIG. 4. *Detailed data from which four of the curves of Fig. 3 were drawn.*

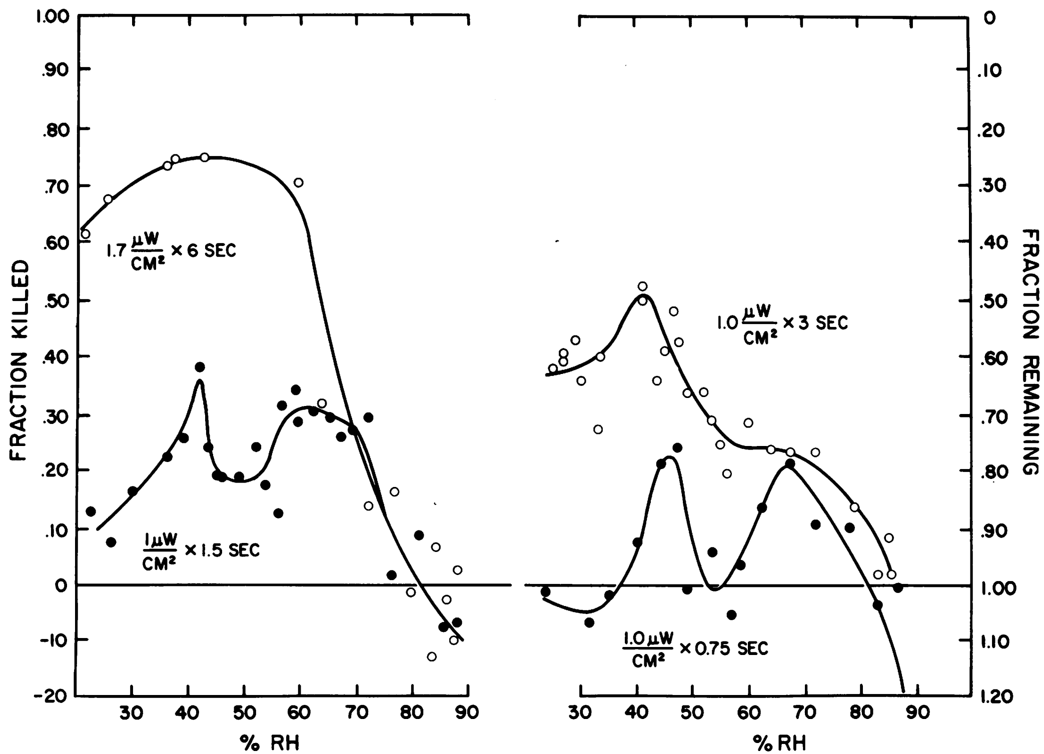


FIG. 5. *Detailed data from which four of the curves of Fig. 3 were drawn.*

complex at low dosages of UV. For example, at 1.7 μw/cm² × 6 sec a decrease in killing at RH of less than 40% is first seen. At 1.0 μw/cm² × 6 sec, this tendency is accentuated. At 1.0 μw/cm² × 3 sec, additional slope changes appear. At the lowest two dosages, major differences in fraction of organisms killed are seen within narrow ranges of RH.

**Semilogarithmic plot of fraction organisms remaining versus UV dose.** Wells discusses at some length the law of mass action and the monomolecular law of reaction velocity as applied to UV air disinfection (21). If UV dose (UV intensity × time of exposure) and number of viable airborne organisms exposed are the participants in a "monomolecular" reaction which renders organisms nonviable, then, according to the law of mass action and the monomolecular law of reaction velocity, a plot of log fraction of organisms remaining versus UV dose should be a straight line described by the following equation:

$$\text{Fraction remaining} = N/N_o = e^{-KD} \quad (1)$$

$$\ln(N/N_o) = -KD \quad (2)$$

where $N$ = number of viable organisms remaining at time $t$; = corrected colony count with UV on; $N_o$ = number of viable organisms at time zero; = corrected colony count with UV off; $K$ = a constant related to the sensitivity of the organisms to killing by UV; $D$ = UV dose = UV intensity × time of exposure.

We replotted the data in Fig. 3 by reading off along a vertical line, representing constant RH, the fraction of organisms remaining at different doses of UV. The fraction remaining was plotted on a logarithmic ordinate and UV dose on a linear abscissa, which, according to equation 2, should give a straight line. A curvilinear relationship was found (Fig. 6 and 7) suggesting nonconformity with the monomolecular law of reaction velocity. The data could be described at all values of RH by an equation similar to equation 2 but with $D$ raised to the ½ power:

$$N/N_o = e^{-KD^{1/2}}$$

$$\ln N/N_o = -KD^{1/2} \quad (3)$$

The curves in Fig. 6 and 7 are drawn by using equation 3 and adjusting $K$ to give a good fit. By conventional curve peeling technique, each curve can be broken down into two logarithmic decay curves which individually conform to equation 2 and whose sum approximately equals the upper curve.

**Semilogarithmic plot of fraction remaining versus exposure time.** To simplify the conditions for testing the monomolecular law of reaction velocity, a series of experiments was performed in which, at constant RH, UV intensity was held constant and only exposure time varied. At 10 μw/cm² and 45% RH, the fraction of organisms remaining was determined five times at each of the following exposure times: 6, 3, 1.5, 0.75, and 0.375 sec. When averages of the five replicates were plotted on semilogarithmic paper, the curve of fraction remaining versus exposure time had a shape similar to that of Fig. 6 and 7 (Fig. 8). With intensity held constant at 1 μw/cm² (data from Fig. 3), a curvilinear relationship was again found (Fig. 8).

**Reciprocity between exposure time and UV intensity: the Bunsen-Roscoe reciprocity law.** The Bunsen-Roscoe reciprocity law states that the amount of disinfection, being dependent on UV dose, is not affected by reciprocal changes in UV intensity and time of exposure (21). To test the validity of this law in our system, the fraction of organisms remaining was determined 20 times at 1 μw/cm² and 6 sec of exposure, and 20 times at 4 μw/cm² and 1.5 sec of exposure. The product of time × intensity was thus the same for each series. RH was held constant at 43 ± 1.5%. The average fraction remaining for the first series was 0.438 and for the second series, 0.469. These small differences were not statistically significant. The findings were thus consistent with the Bunsen-Roscoe reciprocity law.

In summary, the data conformed to equation 3 under all circumstances, but this equation was empirical and appeared not to be compatible with the monomolecular law of reaction velocity. On the other hand, the Bunsen-Roscoe reciprocity law was found to hold.

## DISCUSSION

**Accuracy of RH measurements.** One of the necessary conditions for determination of RH by the wet and dry bulb method is that the velocity of airflow passing the wet bulb be high enough to give maximal evaporative cooling and hence a minimal wet bulb reading. To confirm the adequacy of flow rate in our system, humidity readings were taken with the flow doubled and quadrupled. Since the average readings were the same at 1 ft³/min as at 2 and 4 ft³/min, we infer that there was no significant systematic error in the RH measurements. The random error, due mainly to limited precision of temperature readings, was small.

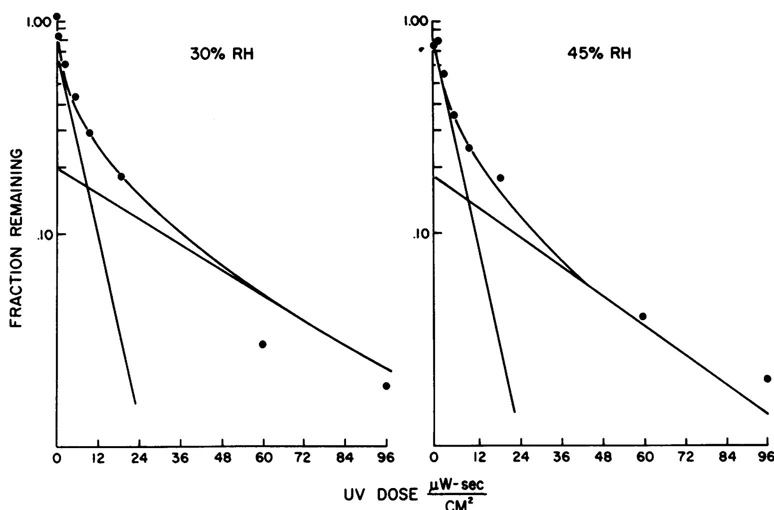


FIG. 6. *Plots of log fraction organisms remaining versus UV dose [(µw-sec)/cm²] at 30 and 45% RH. Curves drawn from equation three with K chosen for best fit with experimental data. Straight lines drawn by conventional curve peeling technique.*

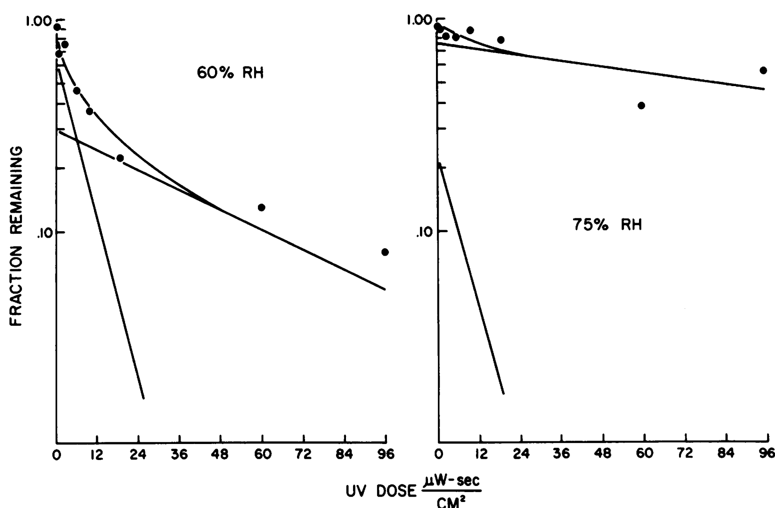


FIG. 7. *Plots of log fraction organisms remaining versus UV dose [(µw-sec)/cm²] at 60 and 75% RH. Curves drawn from equation three with K chosen for best fit with experimental data. Straight lines drawn by conventional curve peeling technique.*

**Accuracy of fraction of organisms killed.** An estimate of the error involved in the determination of the fraction of organisms killed can be made from comparison of replicate determinations. Each determination is a ratio based on the number of organisms remaining with UV on divided by the number of organisms with UV off. The standard deviation of this ratio is ±0.028 for 53 replicate determinations at 43.5% RH. Thus, the scatter to be expected for readings on the ordinates of Fig. 3–5 is 0.028 both for fraction killed and for fraction remaining.

**Monomolecular law of reaction velocity.** When the data failed to conform to equations 1 and 2 and did fit equation 3 reasonably well, we at first doubted that the monomolecular law of reaction velocity applied. It was then realized that the findings could be made compatible with the monomolecular law by postulating more than one family of organisms with different sensitivities to UV, each individually obeying the monomolecular law. Our data are consistent with the presence of two such fami-

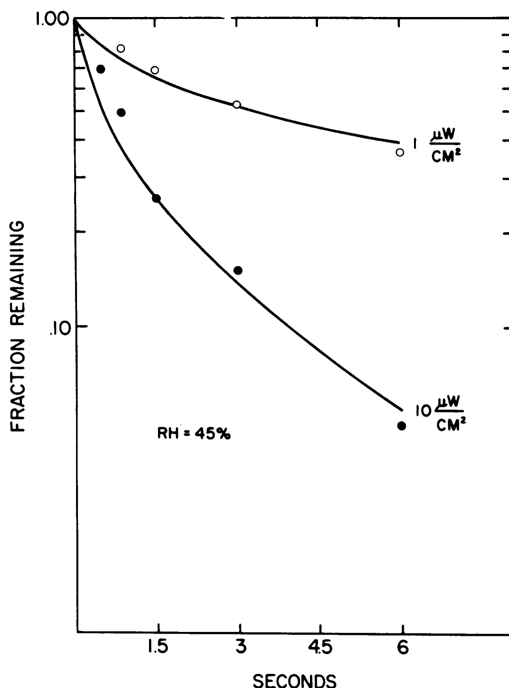


FIG. 8. *Plots of log fraction organisms remaining versus time (seconds) at UV intensities of 1 and 10 μw/cm², with constant RH of 45%. Curves drawn from equation three with K chosen for best fit with experimental data.*

lies, each with a logarithmic decay curve, as described by the following equation:

$$N/N_o = F_1e^{-K_1It} + F_2e^{-K_2It} \quad (4)$$

where $F_1$ and $F_2$ = fraction of organisms in families 1 and 2, respectively, and $K_1$ and $K_2$ = constants defining the UV sensitivities of families 1 and 2, respectively; $I$ = intensity of UV.

From the descriptive point of view, the data are consistent with both equations 3 and 4. Having no reason to doubt the monomolecular law of reaction velocity and having solid precedent for differences in sensitivity to UV among organisms of the same strain (7, 14), we believe that equation 4 gives a better representation of the biophysical processes that occurred. In analyzing the data, we used equation 3 for drawing smooth curves through the data points and equation 4 for deriving the interpretations which follow.

**Effects of RH upon the sensitive and resistant families.** When equation 4 is used, the intercepts on the Y axis give values for $F_1$ and $F_2$, the fractions of organisms in families 1 and 2. It appears that this distribution (Fig. 6 and 7) is affected to an important degree by RH (Table 2).

High humidity caused a larger fraction of organisms to be represented in the UV-resistant family. In addition, the degree of resistance of the resistant family increased with increasing RH as indicated by a progressive flattening of the slope of the decay curve. By contrast, the slopes of the curves for the sensitive organisms remain relatively constant at humidities between 30 and 75% (Fig. 6 and 7).

**Comparison of RH effects with previous studies.** Our primary observations on the relationship between RH and UV sensitivity (Fig. 3) confirm and extend the findings of Gates (5) and of Whisler (22) which showed a precipitous loss of UV sensitivity at RH values above 60%. Our data are not compatible with the work of Rentschler and Nagy who found no RH effect (13). These workers all used *Escherichia coli* aerosols. Phillips and Hanel, using an exposure apparatus more comparable to ours, found no RH effect on the UV sensitivity of *Bacillus subtilis* spores (12). Nowhere have we found reports in the literature of a decrease in the fraction of organisms killed at low humidities. However, at RH values below 40% this effect is clearly shown (Fig. 3-5) for UV dosages below 1.7 $\mu$w/cm² $\times$ 6 sec or (10.2 $\mu$w-sec)/cm². In the middle ranges of RH, our organisms were much more sensitive to inactivation by UV than organisms studied by others (6, 10, 12, 20, 21).

**Reactivation of airborne organisms at very high RH.** The reactivation of airborne organisms which we observed at very high humidities was not photoreactivation since our organisms were not exposed to light after their exposure to UV. The petri plates were incubated in the dark. Since more colonies were counted when the aerosol had been exposed to UV than when it had not, it is difficult to escape the conclusion that the reactivation was caused by the UV. The phenomenon we observed may have been the radiation protection

TABLE 2. *Fraction of organisms in resistant and sensitive families at different values of relative humidity*

| Relative humidity (%) | Fraction of organisms in | |
|---|---|---|
| | Resistant family | Sensitive family |
| 30 | 0.20 | 0.80 |
| 45 | 0.185 | 0.815 |
| 60 | 0.30 | 0.70 |
| 75 | 0.77 | 0.23 |

effect described by Beebe and Pirsch (2) or may have been related to the reactivation phenomenon described by Dimmick (4). The complexities of the lower two curves of Fig. 3 may reflect simultaneous inactivation and reactivation processes in two different strains of organisms at low doses of UV.

**Natural death of S. marcescens versus RH.** The rate of natural death of airborne *S. marcescens* in relation to RH has recently been studied in a rotating drum by Lighthart et al. (8). They found a reduction in natural death rate with increasing RH, reaching exceedingly low values at humidities above 80%. The parallel decrease in natural death rate and in death due to UV at very high humidities emphasizes the importance of water to the survival of airborne organisms.

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