Response of Air-Borne Species of *Pasteurella* to Artificial Radiation Simulating Sunlight Under Different Conditions of Relative Humidity

J. M. Beebe and Gerda W. Pirsch

Chemical Corps, Fort Detrick, Frederick, Maryland

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The bactericidal effect of sunlight was demonstrated as early as 1878 by Downes and Blunt who reported that culture media exposed to sunlight for several hours remained sterile, while corresponding portions of the same media which were allowed to stand in shaded portions of the same room became contaminated. Tizzoni and Cattani (1891) and Wesbrook (1896) reported the killing of *Clostridium tetani* spores by sunlight, while Arloing (1885a, b, c) and Buchner (1892) observed that microorganisms in general were killed by sunlight. Ward (1892) was one of the first investigators to suggest that a specific region of the solar spectrum was responsible for the lethal effects of sunlight. He found that bactericidal activity was a function of the shorter wave lengths, that is, the blue and violet end of the spectrum, and that this activity remained evident even after screening out the yellow, orange, and red bands with suitable filters.

Much recent work in this field has been concerned with the bactericidal effects of the mercury vapor arc lamp emission which is characterized by a highly bactericidal band at 2537 A. This wave length is much shorter than any of the solar radiations reaching the surface of the earth. A few investigators, Buchbinder (1942), Buchbinder *et al.* (1941), Blum and Mathews (1952), and Hollaender (1943) among them, have reported on the action and effects of natural sunlight on agar and aqueous suspensions of a variety of bacterial species. Whisler (1939), working with various sources of artificial light, all of which emitted strong bactericidal bands of wave lengths shorter than 302 mμ, observed that bacteria suspended in air were killed at rates proportional to the intensity of the light, but that the killing effect was modified by, and dependent upon, the per cent relative humidity of the aerosol.

Preliminary work at this laboratory suggested a fairly vigorous bactericidal activity in the solar ultraviolet spectrum (3000 to 4000 A), and the presently discussed series of experiments was designed to furnish some bases for evaluating the effects of sunlight on bacterial aerosols. Inasmuch as the organisms employed for this work, *Pasteurella pestis* and *Pasteurella tularensis*, were both highly virulent, cloud studies were carried out in the laboratory where suitable conditions of safety could be maintained. This restriction necessitated the substitution of artificial simulated sunlight for natural sunlight. While the use of artificial light allowed accurate control of intensity, the composition of the light was somewhat different from that of sunlight, especially in the visible portions of the spectrum. However, the use of filters in combination with the light source resulted in a moderately close approximation to natural sunlight, particularly with regard to bactericidal efficiency. Humidity, a determining factor that appears to be closely linked to the bactericidal action of sunlight, was readily controlled.

Materials and Methods

Cultures. Pasteurella pestis cultures were grown in beef heart infusion broth to which 0.5 per cent xylose was added. Cultures were incubated at room temperature for 24 hr during which time they were shaken continuously at a rate of 72 cycles per min. At the end of the incubation period, the cultures contained approximately 1×10^{10} viable cells per ml. *Pasteurella tularensis* cultures were grown in a casein partial hydrolysate medium at 37 C. Viable cell counts after 20 hr incubation were about 2×10^{10} cells per ml. All cultures were held at 4 C. It was found that long storage, even at this temperature, resulted not only in decreased viability but in a noticeable increase in variability of responses of the cells to treatment. Cultures which, after a period of storage of 10 to not more than 28 days, showed a drop in titer to 1×10^9 viable cells per ml, or less, were discarded. Under certain conditions of light intensity and humidity which appeared to favor growth and made plate counts difficult and inaccurate, it was found necessary to dilute the spray suspension before generation of the cloud. In the case of *P. tularensis*, peptone-cysteine broth was used as the diluent, while beef heart infusion was employed for *P. pestis*.

Chamber. The cloud chamber (figure 1) employed for this work consisted of a glass cylinder (*7*)[1] approximately 28 cm in diameter and 33 cm long, with a vol-

[1] Italic numbers in parentheses refer to numbered items in figure 1.

ume of about 20 L. A Vaponefrin Nebulizer[2] (*6*) and an air inlet tube entered the closed end of the chamber through a rubber stopper. A small rubber bladed fan (*9*) rotated by means of a shaft through a mercury seal at 100 rpm was located at the bottom of the chamber, and served to mix, or stir, the cloud during the spraying period. An exhaust pipe (*11*) led from the top of the chamber to an ultraviolet air sterilizer (*12*), a series of 1½ in. diameter aluminum tubes containing tubular UV mercury vapor lamps (Miller *et al.*, 1955). The total length of this unit was 12 ft. Tests have shown it to be highly efficient in killing not only vegetative cells but bacterial spores passed through at rates of from 1 to 10 cfm.

To allow for irradiation of the cloud, the open end of the cylindrical chamber was covered and sealed with a polyethylene diaphragm (*10*) 0.0015 in. thick. This was transparent to wave lengths as short as 2537 A, and transmitted more than 90 per cent of wave lengths 3000 A and longer.

Humidity of the chamber atmosphere was controlled by varying the moisture content of the secondary air supply (*2*) and (*3*), and the per cent RH was measured by means of an Aminco Hygrometer[3] (*5*) the sensing element of which was incorporated in the common air line. The nebulizer was operated at 12 psi (*1*) and at this pressure the air flow rate through it was 6 L per min, the liquid feed rate 0.2 ml per min.

Light source. The light source used for the irradiation of aerosol clouds was composed of a bank of 16 General Electric Type RS Sunlamps[4] (*13*) mounted on a movable rack, in combination with a plastic filter (*14*) which cut off wave lengths shorter than 3000 A. This filter was developed by means of biological comparisons of natural sunlight with the artificial light of Type RS lamps. These lamps emit a spectrum which is fairly similar, but not identical, to that of sunlight. Since the ultraviolet portion of the spectrum is produced by a mercury vapor arc there is, in spite of the filtering action of the glass bulb, some emission of wave lengths shorter than 3000 A. To approximate natural sunlight, it was necessary to eliminate these. A number of plastic and glass filtering materials were tried but polyethylene film appeared to offer the most promise. Agar plate cultures of *Serratia marcescens* were used as biological indicators in the comparison of natural and artificial sunlight. Inoculated plates were exposed to sunlight of various intensities for periods of time rang-

[2] Vaponefrin Co., Upper Darby, Pennsylvania.

[3] American Instrument Co., Silver Spring, Maryland.

[4] General Electric Co., Nela Park, Cleveland 12, Ohio.

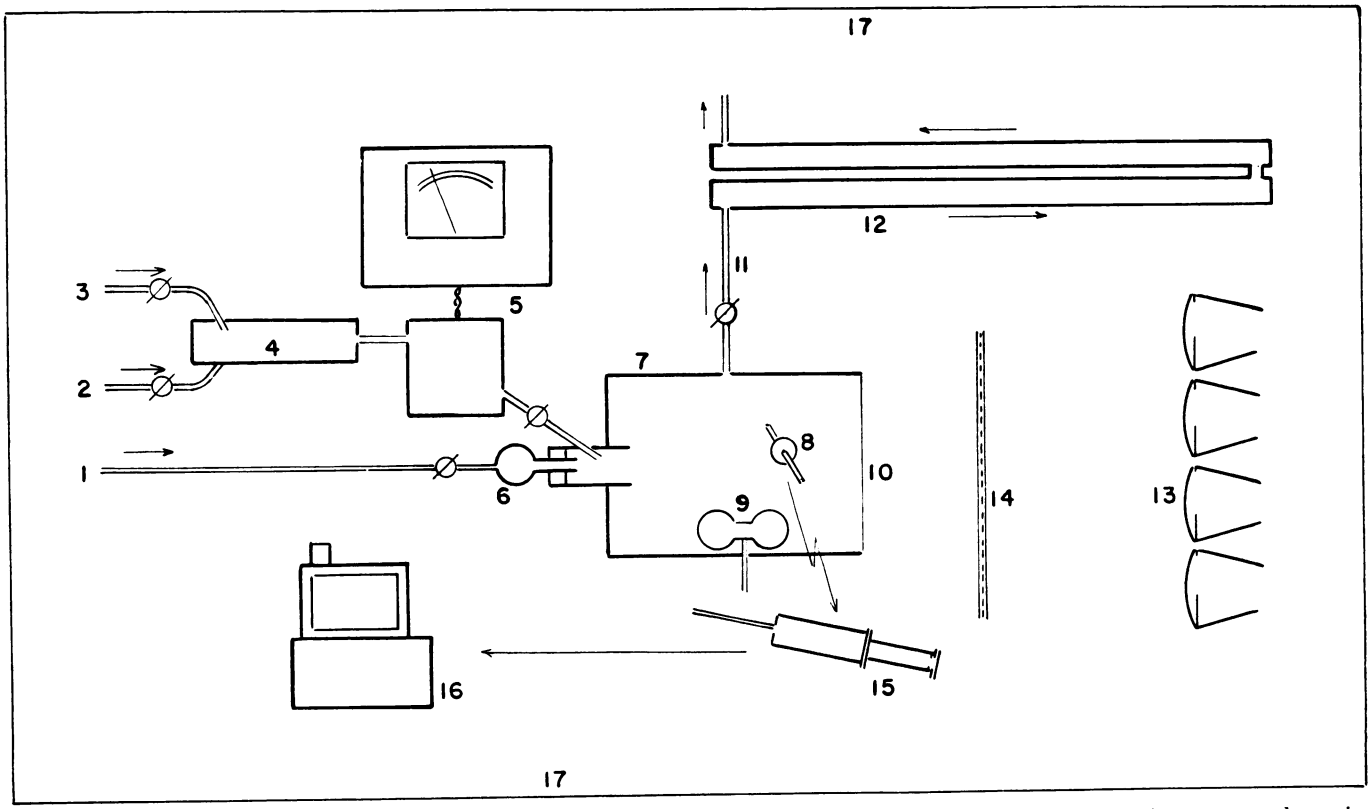

Figure 1. Solar type radiation studies. Schematic diagram of apparatus. *1*, Primary air; *2*, secondary air, dry; *3*, secondary air, wet; *4*, mixer; *5*, hygrometer; *6*, nebulizer; *7*, 20-L chamber; *8*, sampling port; *9*, fan; *10*, polyethylene diaphragm; *11*, exhaust; *12*, ultraviolet air sterilizer; *13*, light source—RS sunlamps; *14*, filter; *15*, 100 ml syringe; *16*, Casella Slit Sampler; *17*, Blickman Safety Hood.

ing from 10 to 90 min. Light intensity was measured in terms of erythemal vitons[5] (the only method available to these investigators at the time), and the dose, intensity × time in min, calculated. Dose was plotted against the per cent survival which resulted in the uppermost curve, labeled Natural Sunlight, as shown in figure 2. About 2000 plates were exposed in this manner.

The experiments were repeated using Type RS Sunlamps without any filters (lower curve, figure 2) and in combination with filters built up of various numbers of layers of polyethylene film. The intensities of the filtered light were adjusted as closely as possible to corresponding sunlight readings. It was found that a filter composed of 32 layers of polyethylene film, each 0.0015 in. thick, allowed transmission of light comparable, from the standpoint of bactericidal efficiency, to natural sunlight at any given intensity. Subsequent tests with two species of *Bacillus* and four of *Pasteurella* corroborated these findings. This combination of sunlamps and polyethylene filter was used for the ensuing work.

[5] Koller (1952) states that, "1 E-viton is the radiant flux which will produce the same erythemal effect as 10 μw of 2967 A radiation. The E-viton. . . is quite analogous to the lumen which is used in photometry." The instrument used in the early stages of this work was a General Electric Sunlamp Test Meter No. 3, which was repaired, and calibrated at 3022 A, by the manufacturer prior to this work.

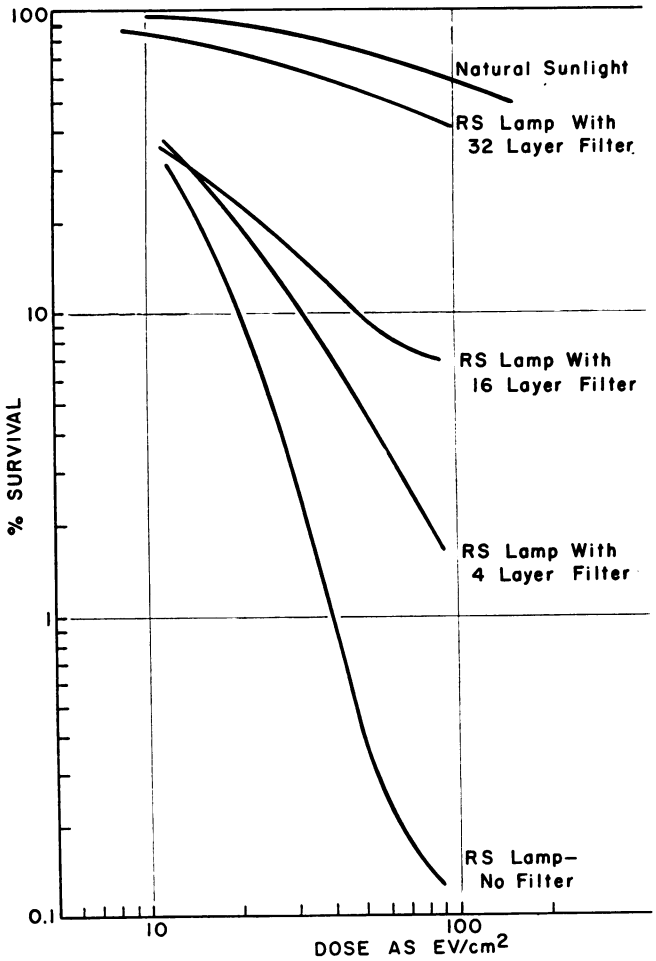

Figure 2. Effect of light on survival of *Serratia marcescens* plant cultures.

Light intensity was controlled by varying the number of lamps and their distance from the chamber, and was measured by means of a radiometer constructed for the purpose. It consisted of an RCA Phototube No. 935,[6] which responded to wave lengths from 2000 to 6000 A with a peak response at 3400 A, and a Corning Glass Filter No. 7-51,[7] which transmitted wave lengths of 3000 to 4100 A, with maximum transmittance at 3600 A. Phototube and filter were mounted in a suitable housing and calibrated at 3650 A by the National Bureau of Standards. The signal was amplified and recorded in microamperes by means of an RCA Ultra-Sensitive Microammeter,[6] and the readings converted to $\mu w/cm^2$.

Sampling. During experimental runs samples were taken routinely from the aerosol cloud at 2-min intervals for the entire period of the run, usually 20 min. Samples were 10 ml in volume, and were obtained with a 100 ml syringe (*15*) fitted with a long, ⅛ in. diameter needle which was inserted through the sampling port (*8*) in the side of the chamber. This port, a rubber tube run through a stopper fixed in the wall of the chamber, was normally kept closed with a pinch clamp. After removal from the chamber the 10 ml samples were diluted with 50 ml of air and plated directly on blood agar by expressing the contents of the syringe into the inflowing air stream of a Casella Slit Sampler[8] (*16*), a device often used for sampling room air. This procedure eliminated the need for resuspension of the aerosol particles in liquid and the necessary subsequent plating techniques. After a suitable period of incubation, 48 to 72 hr, plates were counted, and the minute decay rate (MDR), which was equal to the sum of physical fall-out plus biological death less the product of physical fall-out and biological death, calculated according to the formula:

$$\text{MDR} = 1 - \text{antilog}\,\frac{\log C_0 - \log C\ 100}{T_0 - T}$$

where

C_0 = plate count at time T_0
C = plate count at time T.

The entire apparatus was inclosed in a large Blickman Hood[9] (*17*) and all operations performed with rubber gloves attached to the ports of the hood. Prior to cloud generation, the humidity in the chamber was

[6] Radio Corporation of America, Tube Division, Harrison, New Jersey.
[7] Corning Glass Works, Corning, New York.
[8] C. F. Casella & Co., Ltd., London, England.
[9] S. Blickman, Inc., Weehawken, New Jersey.

adjusted to a previously determined level somewhat lower than that finally desired. After a spray period of 10 sec, during which time approximately 0.033 ml of suspension (containing roughly 3×10^8 cells) was sprayed into the controlled atmosphere, sufficient moisture was added to bring the per cent RH up to the required level. The fan was operated at 100 rpm during the 10-sec spray time and for a period of 30 sec following in order to allow the cloud to become evenly distributed throughout the chamber. The fan was then stopped and the first sample taken. This was considered the initial zero-time sample. Irradiation was started immediately after this first sampling and continued throughout the duration of the run. Samples were taken at 2-min intervals during the entire 20-min run and temperature, humidity, and light intensity were recorded before and after each experiment.

RESULTS

Pasteurella tularensis. Table 1 is a summary of the data obtained under the previously outlined experimental conditions. The number of runs for each combination of light and humidity varied from 6 to 18 with an average of 9.6 runs for each combination of conditions. Total decay rate, the sum of the physical fall-out and biological death less the product of physical and biological decay, and expressed as minute decay rate (MDR), was thought to be the best index of the effects of the combined irradiation-humidity factors on the air-borne bacteria. It should be remarked that physical fall-out was of a low order, since the mass median diameter of the particles generated by the Vaponefrin Nebulizer[2] was about 1.2 μ. Particles of this size, with the density of water, settle at a rate of approximately 5.5 in. per hr. It was supposed that at high humidities, particles might tend to pick up additional moisture which would increase the physical decay rate. This was not borne out, however, by the data obtained during this work. The figures in column 15 for the nonirradiated controls indicate low MDR values at both ends of the humidity scale, 7 and 5 per cent per min at 25 and 95 per cent RH, respectively. The peak decay rate of 18 per cent per min at 60 per cent RH seems to suggest this as a least favorable humidity as far as cell viability was concerned. Decay rates of 15 and 12 per cent per min were obtained at 40 and 80 per cent RH. On these bases it might well be assumed that very high and low humidities favored air-borne cell viability, that physical fall-out throughout the entire humidity range was probably no greater than 6 per cent per min, and that any increase above this figure was the result of biological death resulting from an unfavorable moisture content of the air.

Clouds irradiated at 30 μw/cm^2 presented an entirely different picture. At a relative humidity of 25 per cent the MDR reached a high of nearly 57 per cent per min, and then dropped, as the humidity was increased, to a minimum around 8 per cent per min at 95 per cent RH. At 60 μw/cm^2 light intensity the decay rate increased to 63 per cent per min at 25 per cent RH, which was followed by a uniform decrease as the humidity was raised to about 25 per cent per min at near-satura-

TABLE 1

Effects of solar-type radiation and per cent RH on aerosol stability of Pasteurella tularensis

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
μw/cm^2	Per cent RH	No. of Runs	Average Plate Counts at Time in Minutes:											MDR
			0	2	4	6	8	10	12	14	16	18	20	
0	25	9	446	367	342	328	292	250	215	156	151	122	104	7.0
0	40	13	318	220	133	79	58	44	37	31	25	17	16	15.2
0	60	18	538	202	124	80	52	39	24	20	13	12	8.11	17.5
0	80	11	—	384	473	392	334	285	173	124	94	67	51	12.0
0	95	7	149	95	113	98	76	69	61	65	63	55	47	5.0
30	25	9	402	13	2.44	0.77	0.11	0.38	—	—	—	—	—	56.5
30	40	11	624	35	9.50	3.40	1.77	0.81	—	—	—	—	—	48.5
30	60	8	747	145	53	32	23	13	—	—	—	—	—	33.5
30	80	6	428	521	459	337	227	195	143	93	74	43	22	13.5
30	95	6	169	148	151	157	132	82	82	65	49	45	35	7.7
60	25	8	304	6.75	2.25	0.50	0.12	0.00	—	—	—	—	—	62.5
60	40	8	288	7.75	3.00	1.50	0.18	0.31	0.06	—	—	—	—	51.0
60	60	10	346	40	18	8.40	2.75	1.35	0.95	0.45	—	—	—	38.0
60	80	7	682	499	334	169	88	45	20	11	6.72	1.85	1.14	30.5
60	95	9	371	417	285	193	87	63	39	18	8.44	5.11	1.66	25.3
95	25	11	285	3.72	1.00	0.13	0.09	0.04	—	—	—	—	—	71.7
95	40	11	236	2.09	0.63	0.31	0.00	0.09	—	—	—	—	—	60.0
95	60	14	210	14	1.07	0.79	0.18	0.04	—	—	—	—	—	58.0
95	80	8	190	98	16	6.00	3.31	1.06	0.31	0.25	—	—	—	40.0
95	95	8	86	67	55	42	13	11	2.75	1.00	—	—	—	33.3

tion, and at a light intensity of 95 $\mu w/cm^2$ a corresponding increase in the MDR resulted, reaching a high of 72 per cent per min at 25 per cent RH and a low of 33 per cent per min at 95 per cent RH.

The above mentioned data have been graphically compared in figures 3, 4, and 5. The data from which the slopes in figure 3 were plotted were not all exponential straight lines; many showed a slight curva-

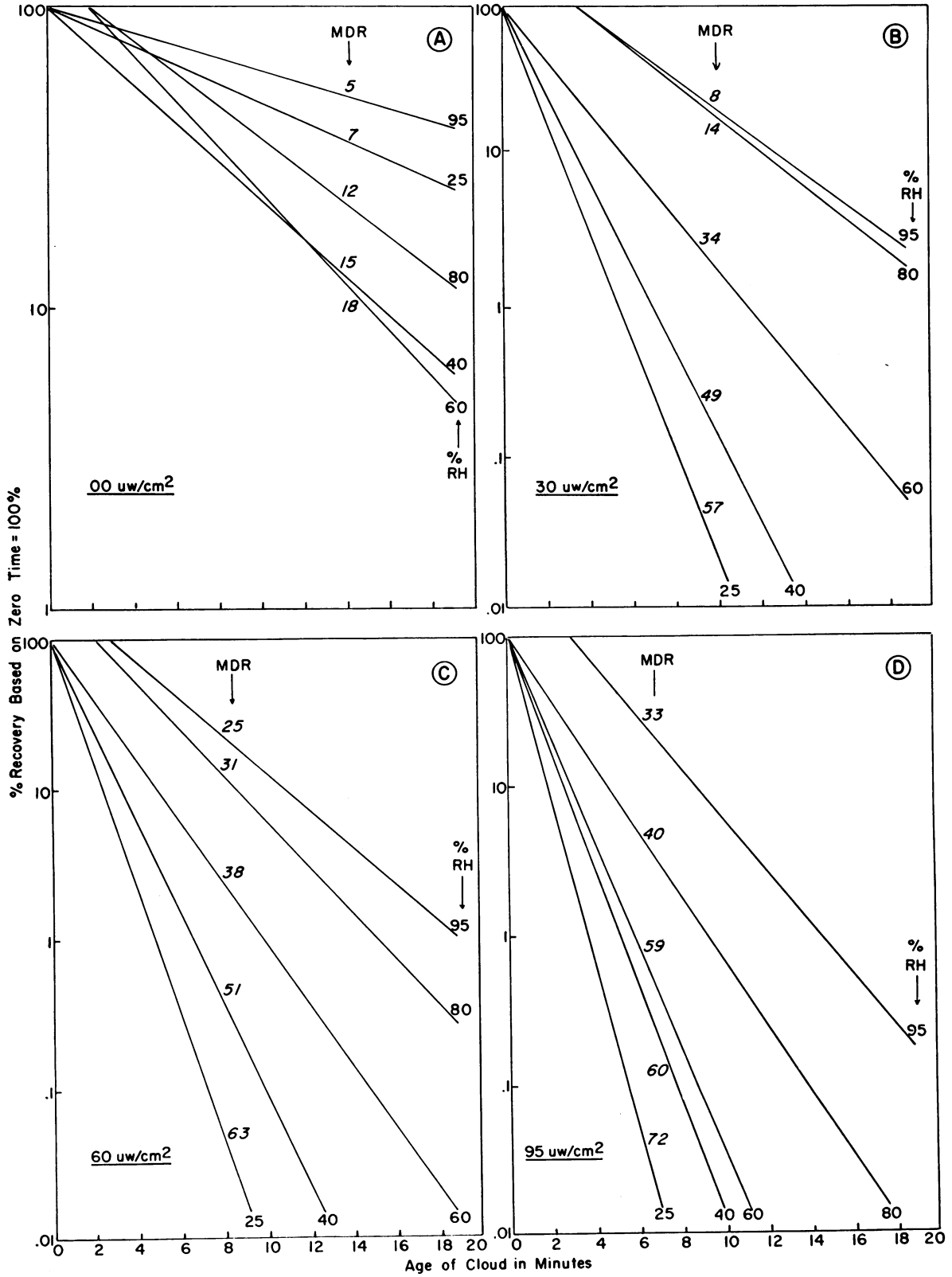

Figure 3. Effect of per cent RH on total decay of *Pasteurella tularensis*

ture. However, the best fitting straight line was, in each case, employed as being representative of the average rate of decay and is so shown in this figure.

In figure 4, the decay rates as per cent per min were plotted against light intensity at five different humidities. This shows the comparative increases in MDR at any light intensity, and demonstrates what appeared to be a protective mechanism associated with the presence of moisture in the atmosphere in which the particles were suspended. The lower curve, that for 95 per cent RH, is comparatively flat at very low light intensities, and increased in rate through the 30 to 60 μw/cm^2 interval. Above 60 μw/cm^2 there is some indication of a slight leveling off. The curve for rates of killing at 80 per cent RH may be seen to follow a more or less parallel course, while at humidities of 60 per cent RH and lower the killing effect of light increased greatly. It is interesting to note that the bactericidal efficiency of low intensity light was proportionately greatest at low humidities.

The protective effect of moisture is illustrated in figure 5 where decay rates were plotted against per cent relative humidity at four different light intensities. The effect of humidity alone on the nonirradiated controls may be noted here, the extremes of the humidity scale producing very low values for MDR, while maximum rates were obtained at 60 per cent RH. Decay rates for clouds irradiated at 30, 60, and 95 μw/cm^2 illustrate the marked degree of protection afforded by moisture. Statistical analyses have shown these decreases in decay rates to be linearly proportional to the relative humidity.

Recoveries based on total number of cells sprayed were low, but for purposes of comparison per cent recoveries based on zero-time counts considered as 100 per cent are shown in figure 3. The zero-time plate counts (table 1, column 4) showed no particular trend, especially those of the nonirradiated controls, although at 95 μw/cm^2 something approaching a definite trend, a decrease in survival with each increase in humidity, seemed evident.

After irradiation was started, definite trends could be seen, and a positive relationship between recovery and humidity made evident. In column 5 it may be noted that at a light intensity of 30 μw/cm^2, recoveries at 25 per cent RH dropped to an average low of 13, but increased rapidly with each successive increase in humidity to a maximum of 521 at 80 per cent RH, with a final drop as humidity approached the saturation point. At the 2-min sampling time, this same pattern was evident at light intensities of 60 and 95 μw/cm^2, and continued to hold at the low intensity throughout the entire course of the runs. However, when light intensity was increased to 60 μw/cm^2 a change in pattern began to manifest itself after about 6 min. For

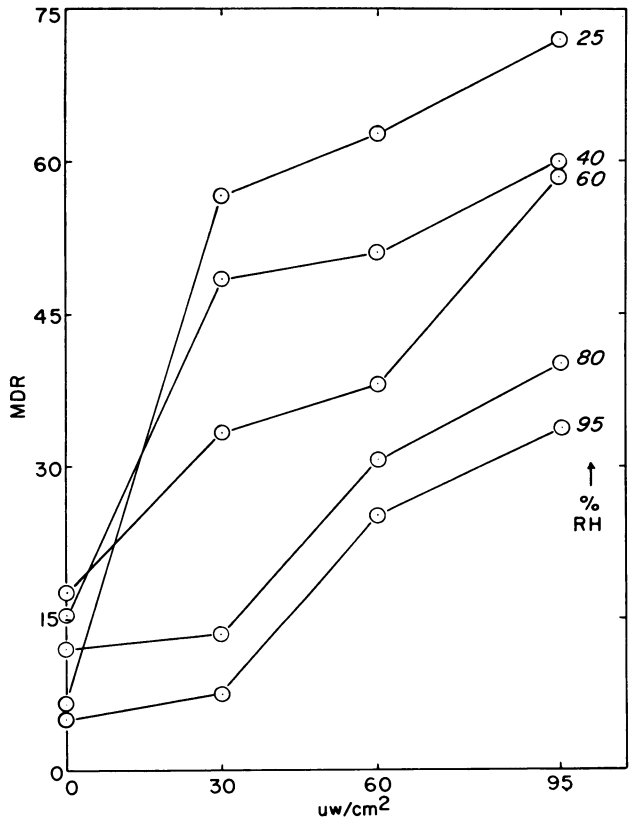

Figure 4. Relative effects of light intensity at five humidities upon *Pasteurella tularensis*.

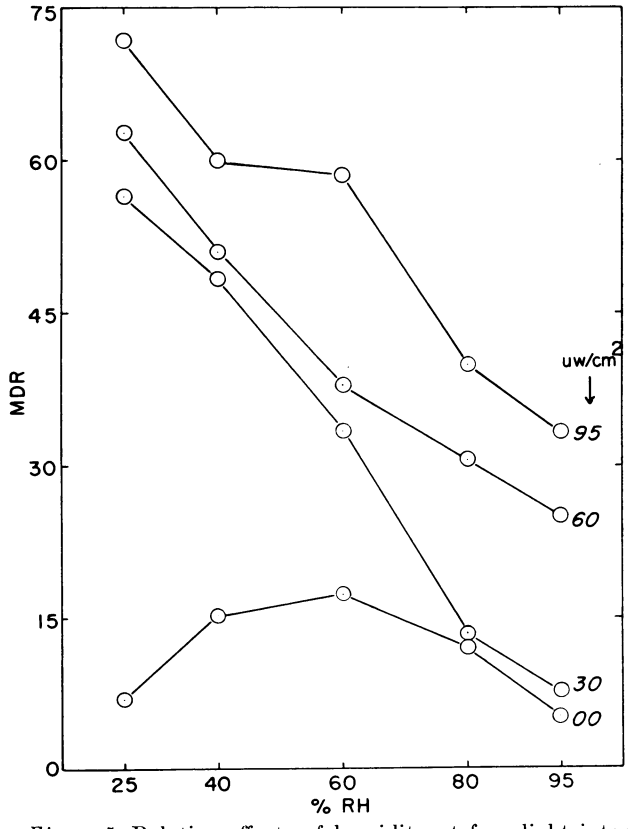

Figure 5. Relative effects of humidity at four light intensities upon *Pasteurella tularensis*.

example, in column 7 it may be noted that peak recoveries were achieved at 95 per cent RH rather than at lower humidities, and this trend continued throughout the balance of all the runs. The same effects were obtained with clouds irradiated at 95 $\mu w/cm^2$.

Pasteurella pestis. The same experimental conditions were employed, and a summary of the data which were obtained may be found in table 2. Minute decay rates for each set of conditions are shown in column 15. The relationships between decay rates and humidity, for the nonirradiated *P. pestis* controls, differed somewhat from the corresponding MDR's obtained with *P. tularensis*. In the present case, a decay rate of 11 per cent per min at 25 per cent RH was followed by a decrease to 8.5 per cent per min at 40 per cent RH. Then, with each increase in humidity, the MDR increased to a maximum of about 24 per cent per min at 80 per cent RH, and leveled off with no further changes irrespective of humidity.

Irradiated clouds showed a reversal of this trend, high decay rates having been recorded at low humidities, followed by more or less uniform decreases in the MDR values as the humidity increased. This general tendency occurred with great regularity throughout all of the work with one exception: clouds irradiated at 95 $\mu w/cm^2$ showed decay rates that decreased with each increase in humidity up to 80 per cent RH, but from this point up to 95 per cent RH a conspicuous increase in the MDR occurred. This was at first thought to be due to experimental error, and a number of additional replicate runs were carried out. However, values for MDR remained high with an average of about 52 per cent per min.

Figure 6 shows the slopes from which the decay rates were calculated. As in the case of *P. tularensis*, not all of the original curves were straight lines, but often slightly bent. In order to simplify calculations, the best fitting straight line, usually a tangent that represented what appeared to be the best average slope, was employed for the determination of the MDR.

Figure 7 illustrates the relative effects of light at the five humidities employed. The uppermost curve, that for 25 per cent RH, shows what appears to be almost a straight line relationship between rate of decay and light intensity. At 40 per cent RH there is a slight tendency for the MDR to level off as the light intensity increased, and this trend was a little more emphasized at 60 per cent RH where the greatest killing effect appeared to be with intensities of 60 $\mu w/cm^2$. The same is true at 80 and 95 per cent RH, with, in addition, a decrease in the rate of killing at the lower light intensity, 30 $\mu w/cm^2$. The fact that the MDR was found to be less in this latter instance than in the nonirradiated controls might suggest the stimulation of growth by small amounts of light energy. A marked variation in the pattern occurred when 95 $\mu w/cm^2$ light produced a great increase in the MDR. The reasons for this are not entirely clear.

The protective effect of moisture is illustrated in figure 8. It will be noted that a humidity of 40 per cent resulted in a lower average MDR value than did 25 per cent RH with nonirradiated clouds, but as the

TABLE 2

Effects of solar-type radiation and per cent RH on aerosol stability of Pasteurella pestis

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
$\mu w/cm^2$	Per cent RH	No. of Runs	Average Plate Counts at Time in Minutes:											MDR
			0	2	4	6	8	10	12	14	16	18	20	
0	25	10	301	158	173	173	133	109	81	64	49	41	39	11.0
0	40	8	213	235	170	159	204	125	91	85	59	56	40	8.5
0	60	8	269	286	212	140	99	56	45	31	19	14	11	16.3
0	80	6	454	221	131	53	30	15	8.17	5.00	5.17	3.83	2.50	23.8
0	95	14	241	233	243	176	236	139	79	44	23	15	6.07	24.0
30	25	9	400	125	73	30	13	3.77	1.88	0.66	0.22	0.22	0.11	37.8
30	40	8	357	146	61	28	11	4.75	2.25	1.37	0.75	0.50	0.12	30.7
30	60	9	180	104	65	44	21	10	6.66	3.44	2.22	1.22	0.77	23.3
30	80	14	158	125	70	41	26	19	14	11	9.00	6.85	4.50	16.0
30	95	6	256	143	331	429	204	147	93	67	48	27	20	17.0
60	25	16	802	337	167	37	7.81	1.62	0.62	—	—	—	—	50.5
60	40	10	654	272	103	25	7.60	1.55	—	—	—	—	—	44.7
60	60	8	339	175	61	18	6.37	1.87	0.25	—	—	—	—	43.3
60	80	8	961	434	169	57	24	10	—	—	—	—	—	36.7
60	95	9	153	92	52	30	12	7.37	—	—	—	—	—	27.0
95	25	6	478	24	4.16	0.33	0.00	0.16	—	—	—	—	—	70.0
95	40	6	420	125	68	12	0.83	0.00	—	—	—	—	—	54.3
95	60	8	116	48	32	17	3.75	0.62	—	—	—	—	—	40.8
95	80	9	93	43	29	13	8.25	3.25	—	—	—	—	—	28.5
95	95	8	82	18	1.75	0.37	0.25	0.12	—	—	—	—	—	51.5

humidity increased, the decay rates showed corresponding increases up to 80 per cent RH. Beyond this point no additional significant increases were noted.

Clouds which were irradiated at 30, 60, and 95 $\mu w/cm^2$ showed the expected increases in MDR values at 25 per cent RH. These values dropped more or less consistently with each increase in humidity, resulting in final decay rates at 95 per cent RH which approached

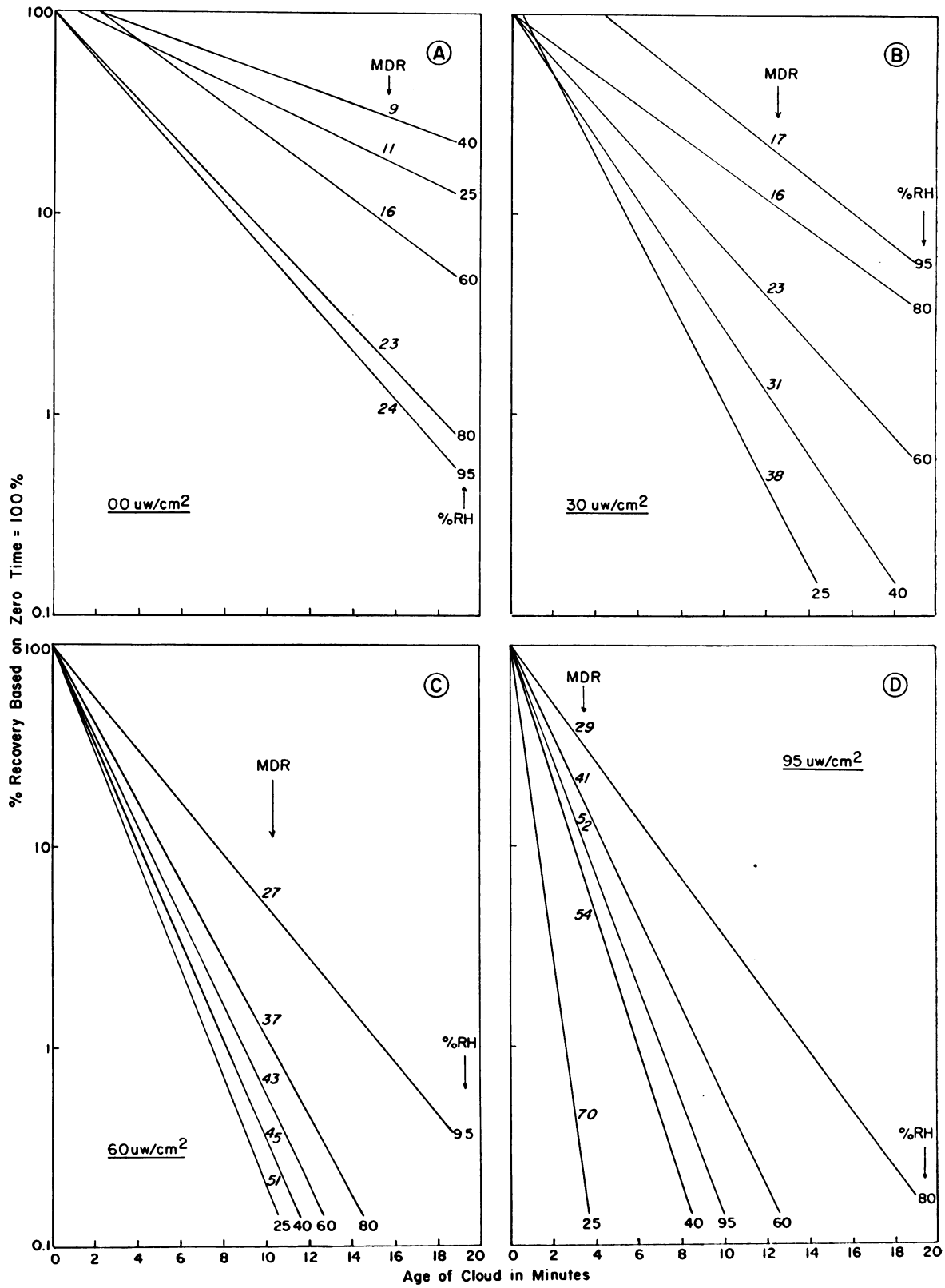

Figure 6. Effect of per cent RH on total decay rates of *Pasteurella pestis*

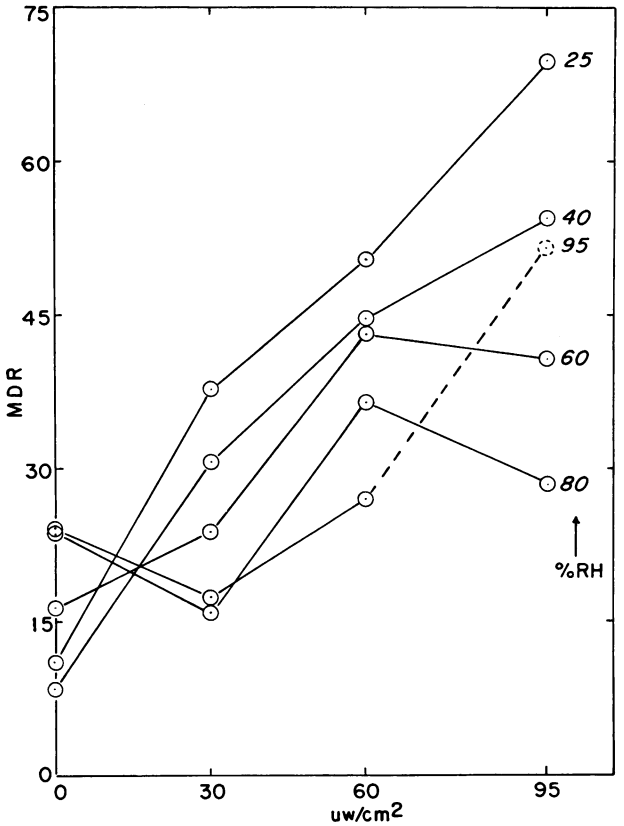

Figure 7. Relative effects of light intensity at five humidities upon *Pasteurella pestis*.

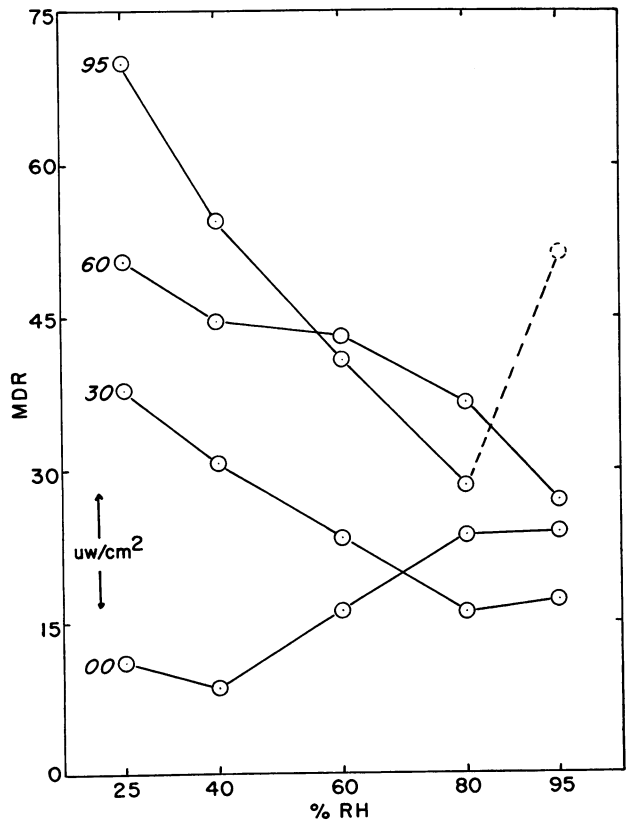

Figure 8. Relative effects of humidity at four light intensities upon *Pasteurella pestis*.

those of the nonirradiated controls. Statistical analyses have shown that decreased values for MDR due to humidity were not linearly proportional to the per cent RH.

Recovery trends (table 2, columns 4 to 14 inclusively) were not well defined until after 6 to 8 min, by which time it was noted that nonirradiated cloud survival, as represented by plate count recoveries, was favored by low humidities. As the per cent RH increased, recoveries decreased up to 80 per cent RH; a slight increase was noted to occur at near saturation. The situation was reversed when clouds were irradiated at 30 $\mu w/cm^2$: recoveries increased with each rise in humidity, maximum survival occurring at 95 per cent RH. Similar results were obtained with light intensities of 60 and 95 $\mu w/cm^2$, although 80 per cent RH, rather than 95, seemed to favor recovery.

Statistical Evaluation and Analysis of Data

Since it is desirable to have an equal number of observations of each treatment, 8 samples of each treatment were randomly selected from the available data. This choice of number required the estimation of 12

TABLE 3

Mean decay rates (MDR) Pasteurella tularensis

Humidity	Illumination ($\mu w/cm^2$)				Mean
	0	30	60	95	
%	%/min				
25	7.0	56.5	62.5	71.7	49.4
40	15.2	48.5	51.0	60.0	43.7
60	17.5	33.5	38.0	58.5	36.9
80	12.0	13.5	30.5	40.2	24.1
95	5.0	7.7	25.3	33.3	17.8
Mean.....	11.3	31.9	41.5	52.7	34.4

95 Per cent confidence limit range for: Over-all MDR, ±1.7 per cent; illumination MDR, ±3.4 per cent; humidity MDR, ±3.8 per cent; and individual MDR, ±7.7 per cent.

TABLE 4

Mean decay rates (MDR) for Pasteurella pestis

Humidity	Illumination ($\mu w/cm^2$)				Mean
	0	30	60	95	
%	%/min				
25	11.0	37.8	50.5	70.0	42.3
40	8.5	30.7	44.7	54.3	34.6
60	16.3	23.3	43.3	40.8	30.9
80	23.8	16.0	36.7	28.5	26.3
95	24.0	17.0	27.0	51.5	29.9
Mean.....	16.7	25.0	40.4	49.0	32.8

95 Per cent confidence limit range for: over-all MDR, ±1.2 per cent; illumination MDR, ±2.4 per cent; humidity MDR, ±2.7 per cent; and individual MDR, ±5.4 per cent.

decay rates for the 7 cases wherein less than 8 samples were taken.

The variation among observations with *P. tularensis* was greater than that of *P. pestis*, so that separate analyses were required for each. Tables 3 and 4 contain the mean decay rates by treatments for *P. tularensis* and *P. pestis*, respectively. Tables 5 and 6 contain analyses of variance.

P. tularensis. Irradiation caused an increase in decay rate. The increase in decay rate was linearly related to the intensity of light (table 5, line 1).

In general, the decay rate decreased linearly with increased humidity. This effect was consistent in the presence of light, but it was not the case when no light was present. In the latter case, a maximum decay rate occurred in the vicinity of 60 per cent RH, with minimum decay occurring with the extreme humidities.

The coefficient of variation among individual decay rates was 16.5 per cent.

P. pestis. Irradiation caused an increase in decay rate, but the relation between light intensity and decay rate was not linear as in the case of *P. tularensis*.

Over the 25 per cent and 80 per cent range of relative humidity there was a linear decrease in decay rate, but the rate increased at 95 per cent RH (table 4). Although the over-all mean decay rates were similar for both organisms, *P. tularensis* showed the greatest range of stability, both with respect to illumination and humidity changes.

The coefficient of variation among individual decay rates was 11.5 per cent.

TABLE 5

Analysis of variance of Pasteurella tularensis decay rates expressed as per cent survival per minute

Line No.	Source of Variation	D.F.*	Mean Square	Error Line	F_0*
1	Illumination	3	1.037289	4	86.32†
	Linear	1	3.056504		>100†
	Remainder	2	0.027681		2.30
2	Humidity	4	0.680945	4	56.67†
	Linear	1	2.684612		>100†
	Remainder	3	0.013056		1.09
3	I × H	12	0.103615	4	8.62†
4	Error	136	0.012017		
		Excluding 0 Illumination			
5	Illumination	2	0.426572	8	27.60†
	Linear	1	0.853052		55.19†
	Quadratic	1	0.000092		<1
6	Humidity	4	0.928932	8	60.10†
	Linear	1	3.621372		>100†
	Remainder	3	0.031453		2.04
7	I × H	8	0.021662	8	1.40
8	Error	102	0.015456		

* D.F. = degrees of freedom; F_0 = variance ratio

† Probability of an "*F*" of this magnitude being due to chance is less than 0.01.

TABLE 6

Analysis of variance of Pasteurella pestis decay rates expressed as per cent survival per minute

Line No.	Source of Variation	D.F.*	Mean Square	Error Line	F_0*
1	Illumination	3	0.686525	4	113.83†
	Linear	1	2.000300		>200†
	Remainder	2	0.029638		4.91†
2	Humidity	4	0.065037	4	10.78†
	Linear	1	0.177190		29.38†
	Remainder	3	0.027652		4.58†
3	I × H	12	0.064102	4	10.62†
4	Error	132	0.006031		

* D.F. = degrees of freedom; F_0 = variance ratio.

† Probability of an "*F*" of this magnitude being due to chance is less than 0.01.

Analysis

Regressions of log-recovery versus time were computed for each sample. The slopes thus obtained were expressible in units of log-per cent survival per min.

Since the standard errors among the sample survival rates decreased with increasing mean survival rates, the "antilogs" of the slopes obtained in the regressions were used for analysis in order to have homogeneous variances. Thus the analyses of variance were performed using per cent survival per min as the unit of measure.

The variances of the two organisms were not homogeneous. Thus a combined analysis was not appropriate and separate analyses were performed.

The means shown in tables 3 and 4 represent the expected per cent reduction of surviving organisms in the aerosol during succeeding minutes.

The linear components of illumination and humidity given in tables 5 and 6 assume equal intervals between the levels of the effects (which was not quite the case) and hence do not give an entirely precise estimation of the variation attributable to these effects.

DISCUSSION

A protective mechanism associated with the moisture content of the atmosphere seems to be instrumental in reducing the bactericidal effect of solar type ultraviolet light. This is not well understood. As Koller (1952) points out, water itself is quite transparent to wave lengths as short as 2537 A, 90 per cent of which will pass through a 3 in. layer of distilled water; and a 20 mm layer of sea water, containing moderately large amounts of dissolved solids, was found to transmit about 90 per cent of 3000 A energy. Longer wave lengths, such as constitute the major portion of the solar ultraviolet spectrum, are transmitted with even greater facility. As opposed to this UV permeability of water, the present investigators have found that a layer

of water not much more than 1 μ thick surrounding a viable bacterial cell suspended in the air, and exposed on all sides to the action of light, might seem to offer nearly complete protection to the cell against the action of simulated sunlight. One possible explanation of this phenomenon might be the presence of dissolved solids in the moisture surrounding the cell. Prior to spraying, the bacteria were suspended in a nutrient material, its composition depending upon the species of bacterium involved. The actual amount of dissolved solids was usually less than 2 per cent but, when added to dead cells and other debris, the total amount of nonviable material in solution and suspension might conceivably be as high as 5 per cent. When a particle first leaves the spray nozzle it is thought to undergo quite rapid and rather drastic changes. Evaporation occurs resulting in a nearly dry particle, and there is a marked drop in particle temperature. At this point the particle probably consists of viable and nonviable cells, cell debris and high concentration of nutrients with very little moisture. It is thought that initial and equilibrium conditions are not necessarily identical; the problems of particle temperature and moisture content under various atmospheric conditions should be much more thoroughly investigated. Then, depending upon the relative humidity of the atmosphere into which the particle was sprayed, moisture is believed to adsorb to, and be absorbed by, the particle. During this period the soluble solids go back into solution and, when a vapor pressure equilibrium is established between the particle and its environment, particle size should be fixed. At near saturation, however, the concentration of dissolved solids is considerably less than at lower humidities, but it might seem possible that a critical concentration of these materials at 95 per cent RH, for example, could be more opaque to the passage of ultraviolet than the same materials in a near dry state.

A second possible explanation should include the condition of the bacterial cell itself. Since the cell's normal environment is liquid, it is possible that it should be more resistant to the action of certain physical forces when suspended in a liquid medium than when in a near dry condition. As opposed to this, however, is the fact of lyophilization for preserving the viability of cultures. Cultures dried under proper conditions are known to maintain a high degree of viability for long periods of time.

What might appear to be a more likely explanation can be condensed in two statements by Fritz (1957): "Small amounts of many substances absorb solar energy, but the principal absorbing mediums are ozone, water vapor, and cloud particles," and "(Albrecht) found large oscillations in the water vapor absorption coefficients as the wavelengths varied. An interesting point is that the coefficient for the vapor often exceeds that of the liquid in the region below 1.5 μ." (1.5 μ = 15,000 A.) If absorption of the bactericidal ultraviolet occurs in the environmental atmosphere, then the size of the particle, and its composition, would have little bearing on the viability of the contained cells, at least as far as the effect of sunlight is concerned.

Temperature is a factor that undoubtedly should enter into a consideration of the responses of aerosolized cells to environmental conditions. The temperature of the atmosphere and the particle suspended in it both appear to be significant. Rather casual observations during the course of the experiments have indicated a fairly definite relationship between air temperature and recovery. At the time of this work, temperature control was not possible, but recorded data indicated that in general, recoveries dropped as air temperatures increased. In view of the fact that rather large amounts of infra red light were emitted by the Type RS Lamps, along with ultraviolet and visible light, this could be a matter of some importance. Decay rates also appeared to be affected by air temperature, although not to the same degree as were recoveries. However, this was an observable phenomenon and apparently plays a part in the responses of air-suspended bacteria to the environment. It is possible that one cause of the great increase in the decay rate during the exposure of *P. pestis* aerosols to high intensity light at 95 per cent RH was the increase in particle temperature. Recorded air temperatures seldom exceeded 39 or 40 C, but during an informal discussion of the problem it was postulated that, under conditions of high light intensity and high humidity, particle temperatures as great as 20 to 50 degrees above air temperature could develop; this might well have a deleterious effect on the cells contained within the particle, and result in both decreased recoveries and increased rates of biological decay.

The possibility of growth stimulation by small quanta of solar ultraviolet light has been discussed to some extent by the authors and co-workers. On the basis of accumulated data there is reason to believe that such stimulation does occur. During early phases of this work plate cultures of a number of species of bacteria were exposed to such varied light sources as germicidal lamps, natural sunlight, incandescent lamps, and sunlamps of two types. In each case where ultraviolet light was emitted, that is, all of the above except the incandescent lamps, low intensities for short periods of time often resulted in survival plate counts as much as 20 per cent higher than those of the corresponding nonirradiated controls. When the dose was increased beyond a certain critical point, however, plate counts decreased with each increase in intensity. Inasmuch as plates were cooled during exposure by being placed on a bed of Sno-Gel[10] at about 10 C it is felt that the possible effects of temperature were minimized, and any

[10] Sno-Gel Co., Oakland, California.

changes that occurred were due to the action of light. A possible parallel example of growth stimulation by normally toxic substances has often been noted by persons using antibiotic assay plates: an antibiotic-impregnated disk on an inoculated plate frequently shows an area of complete inhibition surrounded by an area of abnormal growth, outside of which normal growth of the culture occurs. It is possible that those instances reported in this paper in which decay rates statistically lower than those of the corresponding nonirradiated controls were not artifact, but were actually the result of the stimulation of some bacterial cells, that, under normal conditions, would not grow.

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SUMMARY

A method for studying the responses of aerosolized bacteria to certain atmospheric conditions is described.

Artificial solar type irradiation caused an increase in the decay rate of *Pasteurella tularensis* aerosols which was linearly proportional to the intensity of the light, and in general the decay rate decreased linearly with increased humidity in the presence of light.

Artificial solar type irradiation caused an increase in the decay rate of *Pasteurella pestis* aerosols, but the increase was not linearly proportional to the light intensity. The decrease in decay rate caused by humidity was linearly proportional over the 25 to 80 per cent RH range, but not at humidities above that.

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