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TECHNICAL STUDY 44

EFFECT OF AIR IONS ON BACTERIAL AEROSOLS

JULY 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL STUDY 44

EFFECT OF AIR IONS ON BACTERIAL AEROSOLS

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FOREWORD

The research reported here represents a preliminary effort to evaluate the possible impact on quantitative aerobiological studies of the air ion content of the testing environment. It is in keeping with a previous report that summarized present-day knowledge on air ion effects and listed a number of possible research applications (Technical Study 40, Feb. 1962).

The authors wish to acknowledge the technical assistance of Professor Dominic Greco and Mr. Sam P. Bailey of Safety Division.

ABSTRACT

The effect of positively and negatively charged air ions on aerosols of Serratia marcescens was evaluated by comparing rates of exponential bacterial decay. Ions of both polarities were responsible for significant increases in the mean exponential decay rates when compared with a non-ionized ambient atmosphere. Negative ion atmospheres were shown to be slightly more active than positive ion atmospheres, which is probably due to a greater biological action of negative ions. Further study of the action of air ions on microbial aerosols under conditions of increased quantitation will answer some of the questions raised by this preliminary study.

DIGEST

Recent experimentation on the biological effects of air ions has demonstrated beyond reasonable doubt that under certain circumstances air ions can cause or are associated with changes that, although not of great magnitude are both quantitatively assessable and reproducible. The studies reported here are a preliminary effort to determine if the magnitude of the effects of air ions on microbial aerosols is sufficient to justify measurement or even control of this phenomenon during quantitative aerobiological studies.

The rate of exponential decay was used for statistically comparing the effect of positive and negative air ions on aerosols of Serratia marcescens and di-sodium fluorescein, singly and in combination, during a 12-minute interaction period. Control tests were carried out without artificially produced air ions. Analysis of the data from 34 complete aerosol trials, each with a positive ion test, a negative ion test, and a control (no added ions) resulted in the following significant findings:

(a) Exponential decay of Serratia marcescens aerosols was two to three times greater in the presence of positive or negative air ions than the decay under the control or ambient conditions. Negative ions affected total decay to a greater extent than did positive ions.

(b) Both the addition of bacterial aerosols to ionized air and the generation of ions in an existing aerosol resulted in significantly greater exponential decay rates compared with those of the controls. Moreover, with negative ions, the longer contact with aerosols obtained by adding aerosols to ionized atmospheres produced significantly greater decays than the later addition of ions to the aerosol. That this result was not obtained with positive ions suggests a basic difference in the nature of the action of the two ion polarities.

(c) Using selective particle size sampling devices, it was shown that the general size of the air-borne particles did not change with aerosol age (up to 12 minutes) or with air ion treatment. In all cases the air-borne particles were less than five microns in diameter. The size of the particles that settled out during the experiments was not determined.

(d) Comparing physical aerosol decay with biological aerosol decay in the presence and absence of air ions showed that most of the increase in aerosol decay with air ion exposure was due to increased physical decay. Positive ions caused no increase in biological decay. Negative ions produced significantly greater exponential biological decay rates than the control or positive ion treatment.

From these results it is concluded that high concentrations of air ions significantly affect air-borne microbial particles. Ions of both polarities will result in significant increases in the exponential rates of physical decay. In addition, negative ions appear to have biological activity in that they will increase the biological component of the exponential decay rate.

Since these experiments were not carried out under ideal conditions, e.g., the temperature and humidity were not controlled, the results should be considered tentative pending further experiments with more refined equipment and techniques. The results do indicate, however, that further study of the action of air ions on quantitative aerobiological systems is warranted.

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I. INTRODUCTION

Current interest in research on air-borne infection and in the technology for experimental aerobiology as illustrated by the recent Conference on Air-Borne Infection[1] emphasizes the importance of control of environmental variables during laboratory studies with microbial aerosols. The environmental factors generally considered as requiring measurement and control in quantitative biological aerosol research are temperature and humidity. To a lesser extent, the effects of light and air pollutants have been considered. The present research constitutes a preliminary effort to evaluate the possible influence of gaseous air ions during experimental studies with microbial aerosols.

Air ions have been defined as electrically charged submicroscopic particles of gaseous or solid matter.[2] Positive ions are created by the removal of an electron from an atom or molecule; negative ions are formed by the addition of an electron. Krueger, et al[3] speak of small air ions as consisting of "single ionized molecules about which cluster from 4 to 12 uncharged molecules."

Since it was first demonstrated in 1899 that charged air particles are responsible for the electrical conductivity of the atmosphere,[4] investigators in a number of disciplines have conducted studies on the influence of air ions on living matter. Claims made by many early investigators, who were hampered by the lack of proper means for generating and measuring air ions, gave rise to much controversy, some of which exists to the present time. During the past decade a considerable increase in air ion research was made possible by the development of adequate instrumentation. From the accumulated weight of these studies there can be little doubt that air ions, when applied in controlled experiments, are responsible for certain reproducible biological and physical changes, although it is generally believed that these changes are of a low order of magnitude.[5] The most convincing evidence of the biological effects of air ions is that developed by Krueger.[6-10] These studies have shown that air ions have a significant and reproducible effect on the ciliary beat rate, the mucous flow rate, and the reaction to trauma of the trachea of laboratory animals. Moreover, these investigators have shown that negatively charged oxygen molecules and positively charged carbon dioxide molecules are probably the mediators of air ion effects.[11] Recent work by this group[8] indicates that effects in the trachea depend upon the ability of positively charged carbon dioxide ions to cause a local accumulation of 5-hydroxytryptamine in the tissue, and the ability of negatively charged oxygen ions, acting on cytochrome oxidase, to accelerate the oxidation of free 5-hydroxytryptamine. Krueger's studies have obvious relations to problems of experimental respiratory infections that are not treated in this paper.

Other recent research on air ions has represented broad interests. Kornblueh and his associates have evaluated negative air ion therapy for patients with hay fever, bronchial asthma, and certain respiratory difficulties[12] and have used negative ion therapy as an adjunct in the treatment of burned patients.[13] Other recent studies on the biological

effects of air ions have included effects on the rate of growth of tissue culture cells,[16,18] blood pH, carbon dioxide combining power of animal plasma,[18] and human work performance and visual reaction time.[17] In most studies the magnitude of the reported changes or effects was not large, although there was rather general agreement that positive ions are associated with harmful or undesirable effects and negative ions stimulate or are associated with beneficial effects. Other research has been concerned with the physics of air ions and their interactions with non-biological air constituents. These have added much to our present knowledge of expected ambient air ion densities,[18] the effects of air ions on inert aerosols,[19] and the effects of aerosols on air ions.[20]

Although a number of authors have reported that air ions affect microorganisms, the only quantitative study to date is that of Krueger, Smith, and Go in 1957.* These investigators measured the survival of Micrococcus pyogenes var. aureus in droplets placed in porcelain microtitration dishes and exposed to air ions at concentrations of 1 x 10^8 ions per square centimeter per second or greater. In the absence of smog, exposure to positive or negative ions increased the death rate of the staphylococci in the droplets, apparently by direct action on the bacteria and by increasing the droplet evaporation rate. In the presence of smog, air ions exerted a protective effect on the bacteria by reducing the droplet evaporation rate and delaying the drop in pH. The experiments also indicated that the action of the air ions on the cells could be partly reversed by exposure to intense visible light.

II. METHODS AND MATERIALS

A. EXPERIMENT DESIGN

Aerosols of Serratia marcescens and di-sodium fluorescein singly and in combination were generated in a 305-liter chamber containing a generator capable of producing negative or positive air ions. The aerosol density was measured at designated intervals during the life of the cloud. Each test consisted of three treatments: negative ions, positive ions, and no added ions. The order of the treatments was randomized throughout all tests and a sufficient number of replicate tests were performed to establish statistical validity. The objectives of the experiments were:

(a) To measure the rate of decay of aerosols in the presence of artificially produced positive and negative air ions as compared with the rate of decay obtained when no ions were added.

(b) To determine whether the following factors affect these rates:

(1) Residual effects emanating from the ion-generating equipment (control test).

(2) Time at which air ions are added to the test atmosphere.

(3) Particle size spectrum of the bacterial aerosols.

(4) Physical versus biological characteristics of aerosol decay.

B. AIR ION GENERATING AND MEASURING EQUIPMENT

A Philco Model RG-4 generator* capable of producing air ions of either polarity and equipped with a small fan was used throughout. The ionizer unit was placed inside the aerosol chamber with its controls and power supply unit on the outside. The maximum output setting was used for all tests. Using the Philco Model ICF-6 ion counter, the approximate maximum air ion concentration in the chamber (without aerosol) was 900,000 per cubic centimeter of air. During all tests the generator fan was used to maintain homogeneity in the aerosol.

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* Philco Corp., Communication and Weapons Division, 4700 Wissahickon Ave., Philadelphia 44, Pa.

C. PRODUCTION AND SAMPLING OF AEROSOLS

The bacterial aerosol generator was a simple two-fluid spray tube capable of disseminating a total of one milliliter of liquid material. Aqueous solutions of 0.1 per cent di-sodium fluorescein or broth suspension containing approximately 10×10^8 viable cells of Serratia marcescens were used to charge the aerosol generator. In some tests a mixture of fluorescein and bacterial cells was used. After aerosol generation (requiring about three seconds), samples of the aerosol were taken at 4, 8, and 12 minutes to determine the amount of fluorescein and/or the numbers of viable organisms air-borne per unit volume of air. Sampling was done with all-glass impingers* (AGI) containing 20 milliliters of sterile physiological saline and operated at a sampling rate of 12.5 liters per minute for one minute. The collecting fluid containing the entrapped microorganisms was assayed for viable cell concentration by preparing serial dilutions and plating samples in quadruplicate on the surface of agar plates. The selective nutrient agar used was Difco Peptone Agar** to which was added 0.001 per cent Actidione*** to inhibit fungal contaminants and 250 micrograms per liter of brilliant green dye to inhibit Gram-positive microorganisms. Fluorescein collected in the sampler fluid was assayed photofluorometrically by comparison with standard solutions and the results expressed in micrograms of fluorescein per milliliter.

Following each test, the microorganisms remaining air-borne were reduced to an insignificant order of magnitude by irradiating the interior of the chamber with a 15-watt ultraviolet lamp**** for five minutes with the mixing fan operating.

D. METHOD OF ANALYSIS

Considerable variation occurred in the concentration of air-borne Serratia marcescens cells obtained during the first sampling period of the various replications. However, since we were primarily interested in comparing decrease of concentration with time, rather than per cent recovery, the statistical analysis was confined to decay rates.

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* All-Glass Impinger Sampler, Ace Glass Co; Vineland, N. J.
** Difco Company, Detroit, Michigan.
*** Upjohn Pharmaceutical Co., Kalamazoo, Michigan.
**** Ultraviolet Lamp, HO-15, Westinghouse Electric Corp., Bloomfield, N. J.

From theoretical considerations, it was expected that the change in aerosol concentration with time would be proportional to concentration, i.e.

$$\frac{dC}{dt} = -kC \quad . \quad . \quad . \quad . \qquad (1)$$

where C = aerosol concentration, t= time, and k= proportionality constant. This gives rise to the model

$$C = C_o e^{-kt} \quad . \quad . \quad . \quad . \qquad (2)$$

where C_o = initial concentration of aerosol. This was found to describe the data extremely well. The exponential decay rate is defined as 100 k, expressed as per cent per minute, where k is taken from the model above.

Taking natural logarithms of Equation (2), we have the linear form

$$\ln C = \ln C_o - kt \quad . \quad . \quad . \quad . \qquad (3)$$

In this form k is readily recognized as the slope of the linear regression of the logarithm of concentration versus time. Discussions of the use of this decay parameter in aerosol studies have been presented by Foster[21] and Palmer.[22]

Over the range of concentrations of air-borne material observed in this study, the decay parameter was independent of initial concentration, thereby permitting valid treatment comparisons to be made on the basis of the exponential decay rates alone. Student's "t" test was used for treatment comparisons.

III. RESULTS

A. RESIDUAL EFFECTS OF ION GENERATING EQUIPMENT (Control Tests)

Since the ion generator with its fan and electrical lead wires remained in the aerosol chamber during all tests, it was necessary to determine if the instrument itself and its energized circuitry affected the decay of aerosols. Tests were done, therefore, under simulated positive, simulated negative, and control conditions with the corona tip of the generating probe covered with a plastic envelope to preclude dissemination of air ions. The power supply and polarity switches were operated in the usual manner so that all circuits were energized up to the probe tip as they would be in the usual experiment. We used the Philco Ion Collector to determine that no air ions were released through the plastic envelope into the aerosol chamber.

Data obtained from six trials, each with random-order treatments, are shown in Table I. No significant differences in exponential decay rates were obtained; therefore, it was concluded that the instrument itself and the energized circuits (not including the probe) would not affect the decay of aerosols in subsequent experiments.

TABLE I. ANALYSIS OF EXPONENTIAL DECAY RATES OF S. MARCESCENS AEROSOLS AS AFFECTED BY RESIDUAL EFFECTS FROM THE ION GENERATOR

Treatment	Exponential Decay Rates, per cent per minute			
	Number of Tests	Mean	Standard Error	95 Per Cent Confidence Limits
No Added Ions	6	20.6	5.84	1.45 - 26.8
Negative Ion Circuitry	6	24.6	7.81	16.4 - 32.8
Positive Ion Circuitry	6	21.1	8.18	12.5 - 29.6

Treatment Comparisons	Computed t	Approx. Probability
No added ions vs negative circuitry	1.01	NS*
No added ions vs positive circuitry	< 1	NS
Negative circuitry vs positive circuitry	< 1	NS

* No significant difference.

B. DECAY OF FLUORESCEIN AEROSOLS

Although the removal of inert aerosols by interaction with air ions has been reported, it was of interest to test the effects in these investigations, using the generation and sampling equipment described. In five replicate tests, with random-order treatments, air ion generation was started five minutes before aerosolization of a 0.1 per cent solution of di-sodium fluorescein. After operation of the aerosol generator, samples of the fluorescein content of the air were obtained at 4, 8, and 12 minutes.

Under the control conditions (no added air ions) the exponential decay rates for di-sodium fluorescein were considerably less than those for S. marcescens aerosols. This was expected because of the biological nature of the latter. The presence of positive or negative air ions in the chamber caused a fivefold increase in the exponential decay rates of fluorescein aerosols that was significant at less than the 0.01 level. There were no significant differences in exponential decay rates between the two ion polarities. The decay rates obtained and their analysis are shown in Table II.

TABLE II. ANALYSIS OF EXPONENTIAL DECAY RATES OF FLUORESCEIN AEROSOLS IN THE PRESENCE AND ABSENCE OF AIR IONS

Treatment	Exponential Decay Rates, per cent per minute: Number of Tests	Means	Standard Error	95 Per Cent Confidence Limits
No Added Air Ions	5	6.3	1.12	4.9 - 7.7
Negative Ions	5	33.1	5.98	25.7 - 40.5
Positive Ions	5	31.9	6.05	24.4 - 39.4

Treatment Comparisons	Computed t	Approx. Probability
No added ions vs negative ions	9.83	< 0.01
No added ions vs positive ions	9.32	< 0.01
Negative ions vs positive ions	< 1	NS

C. DECAY OF SERRATIA MARCESCENS AEROSOLS

The effects of air ions on the total decay of air-borne bacterial cells were estimated by analysis of data obtained from 18 replicate trials, each with three air ion treatments arranged in random sequence. In these tests, as in the fluorescein tests, generation of air ions was begun five minutes before creation of the S. marcescens aerosol. Although from day to day there was considerable variation in the initial concentration of aerosol produced, probably because of temperature, relative humidity, and other variations that could not be controlled in the aerosol chamber, conduct of all three treatments during each day provided a basis for comparing the data obtained. Several trials were discarded in which the results of one of the three treatments on one day were lost because of malfunction of equipment.

It was evident from the results that the decay of S. marcescens aerosols was more rapid in the presence of artificially generated air ions of either polarity than in their absence. Table III shows the mean viable cell concentration in the aerosol at the 4-, 8-, and 12-minute sampling times for the three treatments. Conversion of the aerosol concentrations from individual tests to exponential decay rates and analysis of the means of the rates, as in previous tests, showed the exponential decays in ionized air to be from two to three times that of the control. This analysis, shown in Table IV, shows that not only were the exponential decay rates in both test atmospheres significantly higher than the control decay rates, but that the negatively charged atmospheres resulted in higher exponential decay rates than positively charged atmospheres. A graphical comparison of the decay of S. marcescens aerosol with time for the three treatments is shown in Figure 1. In this illustration the derived k values were used, taking the initial recovery as 100 per cent.

D. INFLUENCE OF TIME OF ADDITION OF AIR IONS

In the previous tests the ionizer was turned on five minutes prior to aerosol generation. It was determined that during this period an equilibrium concentration of air ions (approximately 900,000 per cc of air) was established. Further, it was postulated that if such an initially high ion level were necessary to obtain the observed results, allowing the aerosol to come to equilibrium before introducing air ions might improve the survival of aerosolized bacteria.

The influence of time was tested in a series of eight tests in which the ionizer was not turned on until immediately after the four-minute sample was taken. A series of ten tests from those in Table IV, which were done in the previous two weeks and in which the ionizer was turned on prior to aerosolization, was used for comparison. Mean exponential decay rates for the two series of tests are shown in Table V. In each series, negative and positive ion treatments produced exponential decay rates higher than the controls, but we failed to demonstrate significant differences between rates in negative as compared with positive ion atmospheres.

TABLE III. MEAN AEROSOL RECOVERY OF S. MARCESCENS IN THE PRESENCE AND ABSENCE OF AIR IONS a/

Treatment	S. marcescens Cells per liter of Air per ml of Aerosol		
	4 Minutes	8 Minutes	12 Minutes
No Added Ions	269,335	122,227	42,597
Negative Ions	63,114	5,363	557
Positive Ions	70,013	7,707	1,238

a. Mean of 18 tests.

TABLE IV. ANALYSIS OF EXPONENTIAL DECAY RATES OF S. MARCESCENS AEROSOLS IN THE PRESENCE AND ABSENCE OF AIR IONS

Treatment	Exponential Decay Rates, per cent per minute			
	Number of Tests	Mean	Standard Error	95 Per Cent Confidence Limits
No Added Ions	18	22.7	7.03	19.2 - 26.2
Negative Ions	18	78.1	31.71	64.3 - 93.8
Positive Ions	18	53.6	6.11	50.6 - 56.7

Treatment Comparison	Computed t	Approx. Probability
No added ions vs negative ions	7.22	< 0.01
No added ions vs positive ions	14.10	< 0.01
Negative ions vs positive ions	3.21	< 0.01

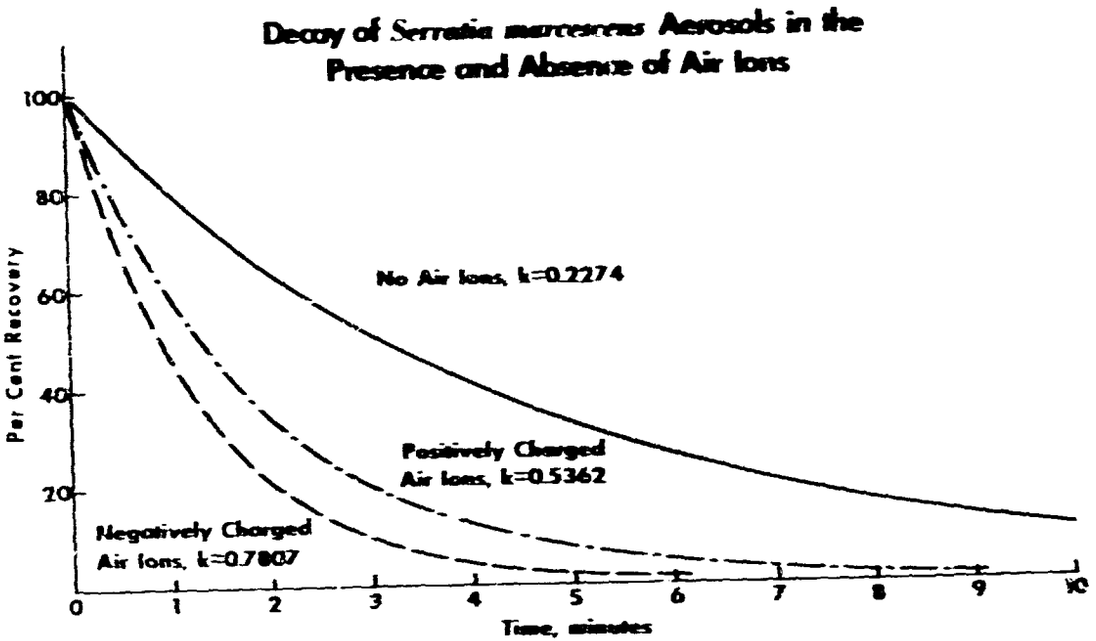

Figure 1. Decay of Serratia marcescens Aerosols in the Presence and Absence of Air Ions.

Comparison of means from the two series, also shown in Table V, indicated that, for both controls and positive ion atmospheres, the influence of time of addition of air ions was negligible. For negative ion atmospheres, however, addition of air ions before aerosolization resulted in a higher exponential decay rate than when air ions were added after aerosolization. Thus it appears that time of addition of the ions is important for negative ions but of little importance for positive ions.

TABLE V. EXPONENTIAL DECAY RATES OF S. MARCESCENS AS INFLUENCED BY TIME OF ADDITION OF AIR IONS

Treatment	Ions Added Before Aerosolization (10 estimates) Mean Exp. Decay Rate, per cent per minute	Std. Error	Ions Added After Aerosolization (8 estimates) Mean Exp. Decay Rate, per cent per minute	Std. Error	Comparison of Means for Time Air Ions Added Computed t	Approx. Probability
No Air Ions	24.5	7.30	20.4	4.37	1.47	NS
Negative Ions	75.6	29.51	50.6	9.50	2.52	< 0.05
Positive Ions	55.4	5.81	55.7	21.69	1	NS

Comparisons	Computed t	Approx. Probability	Computed t	Approx. Probability
No Air Ions vs Negative Ions	5.32	< 0.01	8.80	< 0.01
No Air Ions vs Positive Ions	10.50	< 0.01	4.51	< 0.01
Negative vs Positive Ions	2.12	NS	< 1	NS

E. DECAY IN RELATION TO AEROSOL PARTICLE SIZE

The aerosol generator used produces particles that are initially smaller than five microns in diameter. The AGI sampler is selective for aerosol particles of approximately 17 microns and smaller. The addition of a pre-impinger to the AGI provides a sampler that is selective for particles of five microns and smaller. Thus, operation of the AGI simultaneously with the AGI plus pre-impinger provides a convenient method of partitioning aerosols into two size ranges. This technique was used in further experiments to determine if the size of the viable particles in the air changed with time during the ionization treatments compared with those in the control. It was hypothesized that if, during ionization, the size of the air-borne particles tended to increase with time compared with the control, increased agglomeration by air ions would result in increased settling and be one mechanism responsible for the increased decay rates. In four tests of three treatments each we sampled the aerosol simultaneously with the AGI and with the AGI plus pre-impinger. The aerosol concentrations from the duplicate samples at each sampling period were not significantly different. Exponential decay rates were also compared (Table VI). There were no significant differences in the rates when the two sampler results for each treatment were compared. It was concluded, therefore, that within the accuracy of the sampling devices, the air-ion-treated aerosols did not differ in particle size range from the aerosols in the control environment for as much as 12 minutes of aerosol life.

TABLE VI. COMPARISON OF EXPONENTIAL DECAY RATES OF S. MARCESCENS IN AIR ION ATMOSPHERES IN RELATION TO PARTICLE DIAMETER

Treatment	Mean Exponential Decay Rates, per cent per minute	
	AGI Sampler, 17 microns and less	AGI + Pre-Impinger Sampler 5 microns and less
No Added Air Ions	17.2	21.6
Negative Ions	89.2	64.5
Positive Ions	52.3	59.2

Treatment	AGI vs AGI + Pre-Impinger	
	Computed t	Approx. Probability
No Added Air Ions	2.10	NS
Negative Ions	1.07	NS
Positive Ions	1.75	NS

F. PHYSICAL VERSUS BIOLOGICAL DECAY

From a comparison of the exponential aerosol decay rates of fluorescein (Table II) and S. marcescens (Table IV), it is obvious that the decay function of the latter can be composed of a biological component (death of the cell) and a physical component (evanescence). The final tests were performed by aerosolizing a mixture of fluorescein solution and S. marcescens culture to allow simultaneous assessment of both ingredients. The purpose of the tests was to obtain an estimate of the proportions of the total decay of S. marcescens due to physical fallout and to biological death. In four replicate tests, the order of the three treatments was randomized and the generation of air ions was begun five minutes before aerosolization. The fluid from each sampler was analyzed first for number of viable cells of S. marcescens and then for fluorescein content. Reduction of viable air-borne S. marcescens as a function of time was taken as an estimate of total decay, the reduction in fluorescein as physical decay, with the difference between the two representing an estimate of biological decay.

The results are shown in Tables VII and VIII. In the control tests (no added ions) about three-quarters of the total decay was due to biological death, biological decay being significantly higher than physical decay. The total decays and the physical decays under the influence of air ions were significantly higher than in the controls. In comparing biological decays, however, no significant increase over the control by positive ion treatment (17.6 vs 27.8 per cent per minute) was noted, although negative ion treatment produced a significantly higher biological decay than the control. But when the combined effects of physical and biological decays in the presence of each ion charge were compared, no significant difference was noted.

These tests show that the major part of the decay of S. marcescens aerosols in the absence of air ions is due to biological decay; in the presence of air ions, a greater relative amount of physical decay occurs. Moreover, there appears to be a selective difference in the biological decay resulting from positive and negative ion exposure, with negative ions having a greater biological effect than positive ions.

TABLE VII. ESTIMATES OF BIOLOGICAL AND PHYSICAL AEROSOL DECAYS IN THE ABSENCE AND PRESENCE OF AIR IONS

Treatment	Exponential Decay Rates, per cent per minute: Physical Decay	Biological Decay	Total Decay
No Added Ions	6.4	17.6	24.0
Negative Ions	27.4	45.7	73.1
Positive Ions	22.7	27.8	50.5

TABLE VIII. ANALYSIS OF DIFFERENCES BETWEEN ESTIMATES OF BIOLOGICAL, PHYSICAL, AND TOTAL AEROSOL DECAY RATES IN THE ABSENCE AND PRESENCE OF AIR IONS

Treatment Comparison	Biological Decay: t	Biological Decay: Probability	Physical Decay: t	Physical Decay: Probability	Total Decay: t	Total Decay: Probability
No Added Ions vs Negative Ions	3.24	< 0.05	2.80	< 0.05	3.41	< 0.05
No Added Ions vs Positive Ions	1.65	NS	3.39	< 0.05	4.60	< 0.05
Negative Ions vs Positive Ions	1.92	NS	< 1	NS	1.55	NS

Treatment	Physical vs Biological Decay: Computed t	Approx. Probability
No Added Ions	3.04	~ 0.05
Negative Ions	1.68	NS
Positive Ions	1	NS

IV. DISCUSSION

Although the results reported here must be considered preliminary until confirmed under more refined test conditions, a basis is provided for further hypotheses, both of a practical and hypothetical nature.

On the practical side, the magnitude of the increased decay rates of bacterial aerosols associated with exposure to ionized air suggests that the possible effects of variations in air ion concentrations in aerobiological research should be considered. Moreover, methods by which air ions may be inadvertently produced in those environments should be considered. For example, air ion generators have been made and used that employ nozzles and spray principles not vastly different from those used with some biological aerosol disseminators.[23,24] It is known that the air ion content of the atmosphere varies within wide limits and is markedly affected by air pollutants industrial gases, motor vehicle exhausts, and other by-products of man's technology.[25] Epidemiologically, insofar as air-borne diseases are concerned, it is important to determine to what extent these varying air ion environments contribute to the survival or destruction of air-borne pathogens.

From a theoretical point of view, the demonstration that negative air ions increase biological death of air-borne bacteria may relate to the many references showing improvement from disease conditions and improved body functions in patients exposed to negative air ions. The effect of air ions on aerosol infectivity should be studied quantitatively.

V. CONCLUSIONS AND RECOMMENDATIONS

The most important conclusion drawn from these studies is that artificially produced air ions will increase to a significant extent the exponential decay rates of aerosols of S. marcescens and di-sodium fluorescein. Exponential decay values, defined as 100k and expressed as per cent per minute, were increased twofold to fivefold by air ions.

Analysis of the 34 aerosol trials, each with positive ion, negative ion, and control treatments, resulted in the following findings:

(a) The energized circuitry of the ion-generating apparatus in the aerosol vessel did not affect the decay of aerosols under the conditions of the tests.

(b) Aerosols of di-sodium fluorescein in the presence of negative or positive air ions decayed at a rate approximately five times that obtained under control conditions. There was no selective difference between the action of negative and positive ions.

(c) Serratia marcescens aerosols not in the presence of added air ions showed exponential decay rates approximately four times greater than fluorescein aerosols without air ions. Under the influence of air ions the exponential decay rates for S. marcescens were increased from approximately 23 per cent per minute for the control to 54 per cent per minute for positive ions and 78 per cent per minute for negative ions. The action of negative ions was significantly greater than that of positive ions.

(d) When the procedure of adding aerosol to an atmosphere already containing air ions was compared with the addition of ions after aerosol generation, there was no difference in decay rates with positive ions. However, with negative ions, ionization of the chamber before aerosol generation resulted in significantly higher exponential decay rates. This suggests that a basic difference exists in the mechanism of action of positive and negative air ions on microbial aerosols.

(e) As indicated by comparison of aerosol samples collected simultaneously with the AGI sampler and the AGI plus pre-impinger sampler, no significant differences were detected in the size of the air-borne particles in the chamber during the trials. The generator produced aerial particles that were predominantly less than five microns in diameter at the 4-, 8-, and 12-minute sampling intervals. Ionization did not change the general size range of the particles in the air during these intervals. The relative diameter of the fallout particles during the various treatments was not assessed.

(f) Partition of total exponential decay into its biological and physical components, by simultaneous assessment of fluorescein and S. marcescens aerosols, showed that most of the increase in total decay brought about by air ions is reflected in the physical decay component. Positive ion treatment did not increase exponential biological decay as compared with control tests. However, in addition to the increase in physical decay, negative ions produced a significant increase in biological decay.

These experiments show that decay of aerosols as a function of interaction with air ions can be delineated in a simple aerosol test facility. The magnitude of the increased exponential decay under the conditions specified in these tests was sufficient to characterize air ions as a parameter possibly deserving control. Although most of the observed increase in decay can be said to be due to the physical action of air ions, there was repeated evidence that negatively charged ions, in contrast to positively charged ions, are responsible for a significant amount of biological aerosol death.

One limitation of this study was the fact that the action of various air ion densities was not tested; in all tests the ionizer was operated at maximum output. Another was the fact that control of the temperature and humidity in the aerosol vessel was not possible.

The possible implications of the action of air ions during aerobiological studies should be further investigated under more precise test conditions and with the use of infectious microbial aerosols. The latter will also allow assessment of any changes in organism infectivity.

LITERATURE CITED

1. "Conference on airborne infection," Walsh Mc Dermott, Chariman and Editor, Bact. Reviews, 25:1-377, 1961.

2. Kornblueh, I. H. "Brief review of effects of artificial ionization of the air and ultraviolet radiation," Intern. J. Bioclimatol. Biometerol. 2: part 4, Section Coe, 1958.

3. Krueger, A. P.; Smith, R. F.; and Go, I. C. "Action of air ions on bacteria," J. Gen. Physiol., 41:359-381, 1957.

4. Wilson, C. T. T. "On the comparative efficiency as condensation nuclei of positively and negatively charged ions," Proc. Royal Soc., 65, 1899.

5. Krueger, A. P. "Air ions and physiological function," J. Gen. Physiol., 45:233-241, 1962.

6. Krueger, A. P., and Smith, R. F. "Effect of air ions on isolated rabbit trachea," J. Exptl. Biol. Med., 96:807-809, 1957.

7. Krueger, A. P., and Smith, R. F. "Effect of gaseous ions on tracheal ciliary rate," J. Exptl. Biol. Med., 98:412-414, 1958.

8. Krueger, A. P. and Smith, R. F. "The effect of air ions on the living mammalian trachea," J. Gen. Physiol., 42:69-82, 1958.

9. Krueger, A. P.; Smith, R. F.; and Miller, J. W. "Effects of air ions on trachea of primates," Proc. Soc. Exptl. Biol. Med., 101:506-507, 1959.

10. Krueger, A. P.; Smith, R. F.; Hildebrand, G. J.; and Mayers, C. E. "Further studies of gaseous ion action on trachea," Proc. Soc. Exptl. Biol. Med., 102:355-357, 1959.

11. Krueger, A. P., and Smith, R. F. "Parameters of gaseous ion effects on the mammalian trachea. A. Duration of effects. B. Minimal effective ion densities," J. Gen. Physiol., 42:959-969, 1959.

12. Kornblueh, I. H.; Piersol, G. M.; and Speicher, F. P. "Relief from pollinosis in negatively ionized rooms," Am. J. Phy. Med., 37:18-27, 1958.

13. David, T. A.; Minehart, J. R.; and Kornblueh, I. H. "Polarized air as an adjunct in the treatment of burns," Am. J. Phy. Med., 39:111-113, 1960.

14. Worden, J. L. and Thompson, J. R. "Air ion concentration and the growth of cells *in vitro*," Anat. Record, 124:500, 1956.

15. Worden, J. L. "Proliferation of mammalian cells in ion-controlled environments," J. Nat. Cancer Inst., 26:801-811, 1961.

16. Worden, J. L. "The effects of air ion concentration and polarity on the carbon dioxide capacity of mammalian blood plasma," Fed. Proc., 13:557, 1954.

17. Blote, L. "An experimental evaluation of man's reaction to an ionized air environment," Proc. Internat. Conf. on Ionization of the Air, Franklin Institute, Philadelphia, Pennsylvania, 16-17 October 1961.

18. Davis, F. K. Jr., and Spelcher, F. P. "Natural ion levels in the city of Philadelphia," Proc. Internat. Conf. on Ionization of the Air, Franklin Institute, Philadelphia, Pennsylvania, 16-17 October 1961.

19. Whitby, K. T., and McFarland, A. R. "The decay of unipolar small ions and homogeneous aerosols in closed spaces and flow systems," Proc. Internat. Conf. on Ionization of the Air, Franklin Institute, Philadelphia, Pennsylvania, 16-17 October 1961.

20. Ruhnke, L. H. "The change of the small ion density due to condensation nuclei and the relation to the extinction coefficient of light," Proc. Internat. Conf. on Ionization of the Air, Franklin Institute, Philadelphia Pennsylvania, 16-17 October 1961.

21. Foster, W. D. "A comparison of five functions for describing biological decay in aerosols," Biomathematics Division, U.S. Army Biological Laboratories, Frederick, Maryland. September 1962. (Technical Study 42).

22. Palmer, V. S. "Comparison of decay parameters derived from exponential model," Biomathematics Division, U.S. Army Biological Laboratories, Frederick, Maryland. December 1962.

23. Madi, E. "Ionization produced from the evaporation of water," Atti accad lincei classe sci fis mat nat, 26:159-165, (CA 32-7813). 1937.

24. Whilby, K. T. "Generator for producing high concentrations of small ions," Technical Report 12, USPHS Grant 8-23, University of Minnesota Dept. of Mechanical Engineering, July 1960.

25. Brody, L.; Gasa, C. T.; and Phillips, C. B. "Bio-psychologic effects of air ions as factors in highway safety," Presented at the 41st Annual Meeting of the Highway Research Board, National Academy of Sciences - National Research Council, Washington, D. C., Jan 9, 1963.