STUDIES ON AIRBORNE MICROORGANISMS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
ATTILA KAZMER STERSKY
1971

This is to certify that the

thesis entitled

STUDIES ON AIRBORNE MICROORGANISMS

presented by

ATTILA KAZMER STERSKY

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science and Human Nutrition

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Major professor

Date April 2, 1971

O-7639

ABSTRACT

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By

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These studies involved investigation of survival of airborne Salmonella newbrunswick under various conditions, and evaluation of a new type of electrode for the control of several species of airborne microorganisms.

S. newbrunswick suspended in buffered distilled water or skim milk was aerosolized into a controlled chamber and the number of survivors per ft^3 of the air sampled by the Casella method was determined by standard plate counts using standard plate count agar. The total count of aerosolized bacteria was determined by measuring the amount of radioactive phosphorous (^{32}P) introduced in the chamber from previously labeled cultures.

K values representing the natural logarithms of slopes of survival curves of S. newbrunswick aerosolized from distilled water for 20 min at 90, 70, 50, and 30%

relative humidities (RH) at 10 C were 0.0112, 0.0191, 0.0330, 0.0346, and at 21 C 0.0141, 0.0288. 0.059, 0.056, respectively. The corresponding D values (decimal reduction time) ranged from 41 min at 21 C and 30% RH to 206 min at 10 C and 90% RH. The lowest D value corresponding to the secondary death rate during the subsequent 70 min was 108 min at 21 C and 50% RH, whereas the largest was 404 min at 10 C and 90% RH.

The rates of death of S. newbrunswick aerosolized in skim milk were not as great at all RH levels and 21 or 10 C than the same species aerosolized in distilled water. The D values ranged from 164 min to 470 min at 90 and 30% RH levels, respectively.

Activation energies of the death process of airborne S. newbrunswick ranged from 3520 cal/mole at 90% RH to 7357 cal/mole at 30% RH and from 8649 cal/mole at 30% RH and 13,360 cal/mole at 90% RH for the initial and secondary periods of death, respectively. The activation energies were always greater for the secondary death rates. Activation entropies had a negative value.

Spot tests showed that sodium, potassium, magnesium, phosphates, amino acids, and simple carbohydrates

were present in the leakage materials released from rehydrated airborne S. newbrunswick. Protein-like substances, determined by spot tests, gave very weak reactions in the few trials conducted. The weight of gradually rehydrated samples of airborne S. newbrunswick showed a sigmoid curve as the RH was increased from 30% RH to saturation. About a tenfold increase of 265 nm absorbing leakage material was observed in the supernatant fluids of airborne S. newbrunswick as compared to supernatant fluids of unaerosolized suspensions. Chromatographic separation of the 265 nm absorbing material on Biogel 2 indicated a molecular weight about that of adenosine monophosphate.

Electron photomicrographs indicated that the unaerosolized S. newbrunswick plasmolyzed after the fixation and embedding processes. Plasmolysis did not occur with the aerosolized and freeze-dried cells. Reduction in number of ribosomes in the aerosolized, rehydrated and freeze-dried samples occurred.

The bipolar-oriented electrical field (BPEF) increased death rates of S. newbrunswick from K_D (death rate) = 0.061 to K_D = 0.083. The physical removal under the influence of the field was increased to K_p (rate of

physical removal) = 0.1165 (control K_P = 0.0655). Death rates of Serratia marcescens were similar under the influence of the field and control, K_D = 0.3303± 0.1664, and K_D = 0.3979± 0.205, respectively. During the influence of the field the decline in total S. marcescens was about twice that in the control chamber (K_P = 1.0909, and K_P = 0.5299, respectively).

Continuously aerosolized S. marcescens, Pseudomonas fraqi, Candida lipolytica, Bacillus subtilis spores, and Penicillium roqueforti were exposed to various voltages of the BPEF, and the viable count reduction leveled off between 14,000 and 20,000 volts with maximum averages of 43, 59, 47, 52 and 37.5% for these organisms, respectively. The reduction was due mainly to deposition of microorganisms on metal surfaces of walls or floor.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science
and Human Nutrition

1971

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to his major professor, Dr. T. I. Hedrick, for his concern for the progress of this work, his critical guidance and his help in the preparation of the manuscript. The author is indebted to Dr. D. R. Heldman for his contribution in analyzing some of the experimental data and for his helpful guidance throughout this study.

The author is also grateful to Drs. R. V. Lechowich and P. Markakis for their encouragement, suggestions, and their permission for use of essential equipment to conduct some of the trials. The author also wishes to thank Drs. F. Peabody and G. Malcolm Trout for their encouragement and critical review of this manuscript.

Sincere thanks are extended to Dr. B. S. Schweigert, the former Chairman, and to Dr. L. G. Harmon, the interim Chairman, of the Department of Food Science for providing an assistantship to make the program possible. Thanks are also extended to the Envitron Corporation for

providing some equipment as well as financial assistance for part of this study.

The author wishes to thank his wife, Magdi, and his mother whose moral support was a major force in the persuance of graduate studies. The typing of this manuscript by Magdi is also appreciated.

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INTRODUCTION

The control of airborne contamination in food processing plants is of paramount importance. One reason is to extend shelf life of the food products and another is to ensure the food product's safety for human consumption.

Contamination of food from air attains special significance because even a small number of bacteria may cause serious outbreaks of diseases and/or spoilage. The long period between packaging and consumption under the present system of mass production and distribution of foods is conducive to multiplication under favorable conditions. Reduction of the problem of airborne contamination of food should be possible by advanced techniques such as those used for aseptic packaging. More information is required to cope with the problems of airborne contamination and then measures to eliminate or reduce the problem should be elaborated.

Salmonellosis has recently received a great deal of attention because of its public health significance.

Each of the over 1,200 serotypes of salmonellae (Marth, 106) are considered potentially pathogenic. Yet, very little is known about the viability of salmonellae in the airborne state. Although two recent reviewers (Bryan, 22; Marth, 106) referred to the possibility of airborne contamination of food products by salmonellae, they did not provide positive proof. Hedrick and Heldman (84) in their survey in dairy plants ascribed the low average of airborne bacteria in milk drying areas to the emphasis on more thorough sanitation to reduce the possibility of salmonella contamination. More concrete evidence has been provided for the possibility of airborne contamination by salmonellae in turkey processing plants by Zottola et al. (167). Less is known about the survival of airborne salmonellae. Various investigators (41) reported that increases in temperature from 28 C to 37 C and in relative humidity (RH) from 15 to 80% increased the death rates of Salmonella pullorum.

The purpose of this study was to reveal some of the factors that might affect the survival and other characteristics of airborne Salmonella newbrunswick and to evaluate a new electronic device developed for the removal of microorganisms from air.

LITERATURE REVIEW

Aerosol studies on some important microorganisms in plant sanitation

Staphylococci, because of their relatively high resistance in the airborne state (141, 142), have been used in comparative aerosol studies with other microorganisms. Their behavior in air also has been investigated (133) because of a special interest in hospital sanitation. Reports on airborne streptococci appear infrequently, but studies on airborne salmonella and shigella are almost non-existent in the literature.

Strasters and Winkler (133) found that an aerosolized No. 1600 staphylococcal strain showed a low decay at 50% RH and an increased decay rate at high RH. However, they found that media, age of culture, and method of aerosolization greatly affected the results. A staphylococcus strain No. 1600 had a K value ($\log_e$ of slope of survival curve) of 0.0085 at 90% RH. Williams (158) stated that staphylococci commonly survived in the dried state for days or even weeks. Williamson and Gotaas (159)

found that the disappearance of aerosolized Staphylococcus albus, expressed as a K value, increased from 0.015 at 40% RH and 75 F to 0.022 at 60% RH, and 79 F. A similar "disappearance" for S. aureus was found. The K value of Streptococcus salivarius increased as the RH increased from 41 to 50%. However, these investigators argued that the increase in the K value was probably due to clumping as the RH of the air increased. Dunklin and Puck (53) sprayed Group C streptococci and staphylococci from a broth medium. They observed a relatively high mortality of these organisms at approximately 50% RH and a greater survival above or below this RH. The mortality of these organisms was found to be less than that of the pneumococci that were previously studied.

Webb (141) observed that S. albus was far more resistant to aerosol death than Serratia marcescens and Escherichia coli at 30 to 32% RH and 25 C. Webb (142) found good agreement between his K values for E. coli and S. marcescens, 0.029 and 0.036 respectively, and data of Ferry et al. (58) which, after conversion to K values, were 0.021 and 0.042, respectively, for the two organisms. Webb (141) reported the following descending order of aerosol stability at 50% RH and 25 C for organisms

aerosolized from distilled water suspension: S. albus, Bacillus subtilis, S. marcescens, and E. coli. Webb (143) investigated the effects of chemical additives in the medium on aerosolized bacteria, and noticed that inositol gave only a small degree of protection to S. albus compared to its aerosolization in pure water, whereas, for S. marcescens, E. coli, and B. subtilis, inositol produced good protection at 30% RH and 25 C. This phenomenon was explained by the natural stability of S. albus in air. S. albus also was shown to have resistance against sodium chloride in the airborne state; these findings were in agreement with those of Dunklin and Puck (53).

Wells and Zappasodi (157) observed that high RH protected aerosolized beta streptococci (Group C) against the lethal effects of propylene glycol vapor, and that calcium chloride dehumidified air produced lethal effects comparable to propylene glycol. Williamson and Gotaas (159) found airborne S. salivarius to be more resistant than E. coli and staphylococci.

Detailed fundamental studies on the properties of airborne salmonellae have not yet been conducted. However, various investigators (41) studied the effects of temperature, RH, and glycol vapor on S. pullorum after short storage periods. They found that the death rate of this organism increased with increase in temperature from 28 to 37 C and with increase in RH from 15 to 80% The relative germicidal power of triethylene glycol on airborne S. pullorum, as on other organisms, decreased as the temperature increased from 28 C to 37 C or as the RH deviated from 45%. The decreases in viability were due to lethal effects of atmospheric conditions and not to the mechanical removal of the organisms, as was indicated by the Tyndall effect.

Generation of aerosols

Green and Lane (70) described three main types of atomizers: a) an aerodynamical atomizer (25) operated by compressed air and other gas that is characterized by a high output and wide range of droplet size; b) a centrifugal type of atomizer in which the liquid is fed into the

center of a rotating entity and produces a characteristic uniform main droplet size (140); c) the hydrodynamic type in which the liquid under pressure is forced through the nozzle and breaks up into droplets whose degree of dispersion depends on the nature of the liquid. Various types of air jet atomizers have been reviewed by Rosebury (122) and Green and Lane (70). The electrostatic and the acoustic atomizers are less well known (70).

Uniformity of an aerosol cloud is an important factor which can be achieved by allowing the spray jet to travel an unimpeded sufficient distance to permit the coarse droplets to settle. Fincher et al. (62) claimed that a settling prechamber with a De Vilbiss No. 40 atomizer produced an aerosol with at least 90% single cell particles, and not more than 10% with two cells per particle.

Dunklin and Puck (53) reported that the lethal effect on aerosolized pneumococci increased at intermediate RH values when the droplet size increased. Goodlow and Leonard's studies (68) on Pasteurella species indicated that large particulate clouds withstood solar radiation better than small particulate clouds. In wet aerosols, RH levels about 70% gave protection against solar radiation.

The vibrating reed type generator (49, 163) was claimed to give uniform droplet size and caused little mechanical damage to the microorganisms. An ultrasonic method was reported (115) to have high output of almost exclusively mono-disperse aerosols with no damage to bacteria. Dry cultures were disseminated by explosion (12, 43, 47, 69). The dry cultures were reported to behave differently when the cells were protected by adhering organic material. However, Dimmick (44) reported similarity between aerosols atomized from liquids and the powdered type: an initial rapid decay followed by the period of slower, secondary decay.

Sampling of aerosols

The following are basic methods for microbial aerosol sampling: liquid impingement, solid surface impaction, filtration, sedimentation, centrifugation, electrostatic precipitation, and thermal precipitation (70). Aerosol samplers have been reviewed by Wolf et al. (160), Batchelor (9), and Green and Lane (70). However, only two types of aerosol samplers are widely used (3), liquid impingement and solid surface impaction by using the

Andersen (1) and Casella (160) samplers. The AGI 30 liquid impinger (135, 160) for assaying viable numbers of airborne microorganisms, and the Stacked Sieve (1) with solid medium for low microorganism concentration have been recommended by Brachman et al. (19).

The liquid impinger was first described by Greenburg and Smith (72) as a dust cloud sampler and was subsequently developed into the raised Porton impinger (107) with sonic impingement velocity. This impinger is characterized by a high particle retention efficiency for particles down to 0.5 μm. The sonic velocity (51) of the particle, necessary for impingement, is achieved by employing a short length of capillary tubing which acts as a critical flow orifice and, in turn, controls the flow rate. The shearing and impaction resulting from the sonic velocity of the liquid impinger was stated (107) to have some lethal effect upon bacteria. George et al. (66) designed the multiple orifice impinger which reduced losses of the impingement liquid and increased the recovery of organisms by reducing effects of prolonged aspiration.

A definite quantity of air is drawn through the impingement fluid in a finite time, in the liquid impingement technique, then an aliquot of the collecting fluid

is plated in melted agar (107, 122, 136). The liquid impinger is suited mainly for relatively concentrated aerosols, or when a long sampling time is required to enumerate low concentrations of airborne organisms (3).

In the slit sampler, which utilizes the solid surface impaction method of sampling, particles from the air drawn by vacuum are impinged on a revolving petri plate containing solidified agar (17). The plate is then incubated for enumeration of colonies. Bourdillon et al. (17) have developed several types of slit samplers. These complex units are delicate, difficult to sterilize, and require specially designed agar collection plates.

Luckiesh et al. (101) developed a portable air sampler and Decker and Wilson (40) developed a slit sampler applicable to field use. Comparison with the sieve type air samplers (52) showed that the slit sampler was 2.4 times more efficient. Kuehne and Decker (95) have shown that extended collection time increased the volume of air per given surface area of agar and this was detrimental to the viability of S. marcescens. The slit sampler was successfully employed for the collection of T3 bacteriophage (36). Several different types of slit samplers have been described by Wolf et al. (160).

Enclosures for aerosols

The simplest aerosol studies are carried out in rooms (71) or chambers (44, 65) or sometimes in a tank (156, 161) which might contain a fan (161) to produce a homogenous particle distribution and to keep the aerosolized microorganisms from settling. A unique storage method was employed by Ferry and Maple (61) who suspended the aerosol in an expandable balloon. Dallevalle et al. (37) constructed a vertically operated dynamic aerosol chamber with a diffusion head (90) for a descending and continuously removed aerosol. This chamber can also be operated without moving the air mass when organisms are evaluated by settling plates. A horizontally operated dynamic aerosol system (85) was intended for short time aerosol viability studies.

A vertical wind tunnel (50) with a rising airflow permits longer studies. By increasing the size (80) of the Henderson apparatus (85) the storage time for the aerosol was increased. This system also permitted the introduction of air with different relative humidities into the aerosol to study the effects of the stress produced by rapid changes in RH. The apparatus giving the

most satisfactory conditions for long range aerosol studies (up to 2 days) is the rotating drum or toroid (67). In this drum the centrally introduced aerosol is dispersed centrifugally by the slow (3-4 rpm) rotation. This rotation prevents excessive settling of particles in the 1 μm range. The effect of light on aerosols can be studied by making the drum from transparent acrylic plastic (145).

Heckly and Dimmick (83) stated that a study of lyophilized organisms has a place in aerobiology because freeze-dried organisms are similar to airborne cells in the respect that they are essentially naked and thus in direct contact with the atmosphere. Silver (129) used glass fibers for the suspension of small droplets to avoid stresses during aerosolization. Webb (144) employed membrane filters to establish the relationship between water content of cells and cellular death. Rountree (125) studied the effects of drying on S. aureus by exposing suspensions of these organisms on pieces of textile to temperatures ranging from 66 to 80 F and RH levels from 42 to 50%. Cellulose fibers (7, 108, 109, 125) and the inner surface of glass tubing (104) were used also to support bacteria during desiccation.

Determination of survival of airborne microorganisms

A decrease in the viable microorganisms in aerosols is due to both biological and physical losses. These two types of losses must be separated and accounted for in order to determine the ratio of survivors versus the total number of microorganisms (3). An estimate of the total count can be obtained by direct microscopic count following impaction of bacteria on glass slides, or by measurement of light scattered. However, the effectiveness of these methods is influenced by the concentration of the aerosols. Slide culture techniques (119) have been employed to enumerate microorganisms recovered directly on agar surfaces (33).

The most widely employed methods for the determination of total numbers of microorganisms in aerosols are tracer techniques which utilize dyes, spores, radioisotopes, and enzymes which are not destroyed by the aerosolizing process. Methylene blue (60) and sodium fluorescein (161) are dyes which have been employed as tracers. The interfering factors in the application of dyes are that they might be toxic with the possibility of toxicity increase occurring during evaporation; the dyes are present

in all parts of the spray suspension and appear in the aerosol not only in the particles carrying microorganisms but also in other particles not containing microorganisms (3).

B. subtilis (var. niger) spores have been employed as tracers by several investigators (77, 78). However, researchers (2, 27, 77) found that some of these spores die when they are aerosolized and, therefore, cannot be used for accurate determinations. In the radioactive tracer techniques the tracer is incorporated into the microorganisms by growth in a medium containing the isotope; therefore, the presence of radioactivity is the indication of the presence of an organism. The most frequently used isotopes in aerosol studies have been ^{35}S (110), ^{32}P (77, 78, 127, 128), and ^{14}C (2). A mixture of labeled organisms and non-labeled test organisms (2, 77, 110, 142) may be aerosolized. In this manner a highly radioactive heat killed organism facilitates the estimation of the aerosol decay of an unlabeled organism which remains unaffected by the disintegration of the isotope (3).

The addition of radioactive tracer to the microbial suspension (79) for aerosolization is similar to that of the dye tracer technique with its inherent disadvantages.

The β-galactosidase in E. coli B has been shown to resist detrimental effects of aerosolization (2). The amount of activity of this enzyme has been related to total numbers of bacteria (4) which have been estimated by light scattering measurement (45, 60, 63). The limitations of this technique during aerosol storage are: 1) its accuracy is influenced by changes in size distribution, 2) it can be used only within a limited range of aerosol densities (77).

Effect of relative humidity

In most aerosol studies the RH is stated, or the study is specifically directed toward this aspect (38). Loosli et al. (100), Williamson and Gotaas (159), and others (41) found that high RH was more lethal than medium or low RH for airborne bacteria. Wells and Zappasodi (157) reported opposite findings. The lack of control of physical losses might have been the cause of discrepancies. Brown (21) investigated the effects of RH, temperature, age, and sodium chloride concentration on aerosolized E. coli. He observed the highest stability for E. coli near 70% RH at 10 C. The survival of this organism was

affected by age only when exposed to high RH levels. Sodium chloride increased the death rate at 50% RH but not at higher RH levels.

Ferry et al. (58) observed a rapid initial decay in the first 0.5 min followed by a slower decay, and a greater viability of E. coli at high RH than at low RH.

Webb (151) suggested that the death of airborne bacteria resulted from movement of water molecules in and out of the cell upon reaching equilibrium relative humidity. He found that an initial rapid kill (within the first second) was followed by a slower death rate. Hayakawa and Poon (82), and Poon (118), observed a high death rate of E. coli in the first 0.5 second which was related to water evaporation with a succeedingly slower death rate. The effect of water content on the death rate of S. marcescens was investigated by Monk et al. (113, 114). They observed that 33% water content was the most lethal.

Webb (151) stated that individual variation in the organism also affected its response to RH. He found that high RH favored the survival of microorganisms in air (S. marcescens, E. coli, S. albus, and B. subtilis). Rosebury (122) found that bacteria from different genera and several viruses were more stable at 70 to 80% RH than

when sprayed into a dry atmosphere. Webb (151) related death rates of bacteria to water content of cells and RH. He showed that the cells must lose about 90% of their water content before death, which occurs when the water content of the cell falls below 30 g/100 g of cell solids. Bateman et al. (11) recognized several zones of water transfer in S. marcescens cells depending upon ambient RH.

In general, investigators (20, 21, 142, 154, 155) did not detect narrow zones of instability of microorganisms with respect to RH. However, abrupt changes in survival with respect to incremental changes in RH have been reported in air and nitrogen (2, 14, 27, 28, 34). Narrow zones of instability were also found in argon and helium atmospheres (30) at higher RH, and high stability at low RH levels. Decreased survival for freeze-dried S. marcescens at high RH was also reported (38, 114). The cell survival was adversely affected by water content according to Kethley et al. (91). Species of airborne Mycoplasma (162) were most sensitive to RH between 40 and 60%.

Hatch and Dimmick (80) investigated the effects of sudden shifts in RH on Sarcina lutea and S. marcescens. They found that aerosols diluted with constant RH or with

air at an RH less than that of the primary aerosol did not affect the death rates of these organisms. However, an increased rate of death was observed when cells were subjected to an RH that increased from 26 to 50%. These findings were substantiated by subsequent experiments (81).

Effect of temperature

There are only a limited number of studies which involved the survival of airborne microorganisms as a function of temperature. Investigators (41) revealed that the death rate of S. pullorum aerosolized from water increased as the temperature of air increased from 28 to 37 C. Williamson and Gotaas (159) found no effect of temperature change from 24 to 30 C on the survival of E. coli, S. aureus, and S. salivarius.

Harper (75) found that aerosolized vaccinia, influenza and Venezuelan equine encephalomyelitis virus survived better at lower temperatures than at higher temperatures. Webb (142) studied the effect of temperature on S. marcescens at 5 C intervals from 0 to 25 C and at -10 C, and he found that the death rate increased as the

temperature increased. Dunklin and Puck (53) found an increase in death rate of pneumococci when the temperature was raised from 14.4 to 33.3 C and the effect was the most pronounced at 50% RH. Kethley, et al. (91) found a positive correlation between increase in death rate of airborne S. marcescens and increase in temperature from -40 to 32 C at RH from 20 to 80%.

Webb (142) related temperature and RH to the death rates of bacteria by employing Frossling's equation (64) which describes the evaporation rate of water droplets at different temperatures. Webb (151) obtained a sigmoidal curve with a considerable scatter when plotting K values vs. RH.

Ehrlich et al. (54) observed lower recovery for S. marcescens, E. coli, and B. subtilis spores at -40 C than at -18 to 24 C after 4 min of aerosol age. Between -18 and 24 C maximum survival was observed for S. marcescens and E. coli. An increase in temperature from 24 to 49 C resulted in significantly reduced aerosol recoveries of the two vegetative organisms. B. subtilis spores survived well between 24 and 49 C. At -40 C the aerosol recovery of all three organisms was consistently lower than at -18 to 24 C.

Effect of other growth conditions

Death rates of airborne microorganisms may be greatly affected by the circumstances under which the culture was grown. Although the majority of studies give a full statement on growth methods, few carry out evaluations on the possible effects of growth conditions. Brown (20) made the following observations: 1) E. coli grown on agar surfaces had the same death rates below 70-75% RH but was more resistant at high humidities than broth-grown cultures; 2) aeration of a culture increased its resistance at high but not at low RH values; 3) young cultures were more susceptible to death in the airborne state at 90% RH; and 4) at RH values below 90% the death rate appeared to be independent of the age of the culture. Brown's data (20) further indicate that the maximal death rate for E. coli occurred during the transition from lag to logarithmic growth phase or very early in logarithmic growth. Goodlow and Leonard (68) emphasized the need to control the conditions of growth and the harvest age of the culture at the time of aerosolization. They have shown that S. marcescens cells in the lag phase of growth are

more resistant to the stress of aerosolization than are cells in the stage of logarithmic growth.

Brown (21) found that young cells of three psychrophilic genera (Achromobacter, Pseudomonas, and Micrococcus) died more rapidly than old cells at all RH. E. coli, depending on age, died at different rates at high humidities only. Young cultures of E. coli were shown to be more sensitive at high RH than old cultures (27).

Effect of composition of spray fluids

Addition of any solute to the spray suspension tends to alter the response of microorganisms to the stress produced by aerosolization. Therefore, a distilled water suspension of bacteria serves as the basis for comparison in most studies. Additives have been added to the spray fluid to improve survival or to study death mechanisms (3). The additives employed in aerosol studies have been amino acids, antibiotics, aromatic compounds, dyes, metal chelating agents, polyhydric alcohols, salts, spent growth medium, and sugars.

Brown (21) found no effect of sodium chloride on death rates of E. coli, Achromobacter, and Micrococcus at high humidities but in all cases increases in death rates were observed near 50% RH. Sodium chloride increased (151) death rates of aerosolized E. coli and B. subtilis at 60% RH but did not affect S. albus.

Webb (141) showed that chloramphenicol containing suspension of cells of E. coli, S. marcescens, and S. aureus, when sprayed into air, provided a greater protection at high RH levels. Inositol was more effective at low RH levels. Webb (143) proposed the hypothesis that inositol acts as water, in the airborne state, so far as the maintenance of the structural integrity of macromolecules is concerned. Webb et al. (153) found that inositol protected air dried S. marcescens against X-ray damage below 70% RH, and there was little or no radiation damage above 70% RH. Webb (149, 150) ascribed the protective nature of inositol to its strategically placed hydroxyl groups which prevent the destruction of RNA and DNA by dehydration during aerosolization of microorganisms. Webb and Dumasia (154) found the i-inositol was the most effective among the chemicals studied in protecting airborne E. coli against X-ray damage. Webb (151), studying

the effects of a great number of compounds on the survival of airborne microorganisms, suggested that strategically placed hydroxyl, amino, and possibly sulfhydryl groups protect vital macromolecules during desiccation.

Webb et al. (155) found that the capacity of air-dried E. coli to produce T1, T3, and T7 phages was markedly protected by inositol at 60% RH and below. The controls showed a decrease in this capacity.

Preincubation with various inhibitors of the electron transport system such as sodium azide, potassium cyanide and 2, 4-dinitrophenol, before spraying was found (14) to increase the aerosol stability of E. coli B considerably, particularly at a low RH. A free radical scavenger, cystamine, was found to increase the viability of E. coli B in air considerably at low RH (14). These chemical compounds are supposedly protecting agents against the deleterious effects of oxygen during aerosolization.

Cox (29) found that for the E. coli Jepp strain good survival at high RH could be obtained by addition of glycerol to the spray fluid, together with a shift in RH value to 100% before collection. At low RH either raffinose or sodium glutamate were better protective agents

than glycerol. Hess (86) found that Mn^{++}, Co^{++}, glycerol, and thiourea offered protection to S. marcescens in air at 40% RH and 25 C. Zimmerman (166) found that minimally penetrable sugars (di-, and oligosaccharides) stabilized S. marcescens at 30 to 47% RH and 24 to 27 C, whereas, freely penetrable sugars (monosaccharides) stabilized cells during freeze drying. Zimmerman (165, 166) found that in addition to di- and trisaccharides, salts of certain organic acids with multivalent cations (Ca^{++}, Mn^{++}, Mg^{++}, Sr^{++}) especially, and with monovalent cations (Na^{+}, K^{+}, Li^{+}) to some degree enhanced stability of S. marcescens. Sodium chloride at 5% and 10% but not at 1% improved the aerosol stability of S. marcescens. He also found that metal chelating agents in low concentrations, and especially in combination with sugars, greatly increased the aerosol stability of S. marcescens. Harper (76) found that the survival of polio and vaccinia viruses was dependent on the chemical composition of spray fluid, and demonstrated that the influence of RH can be eliminated or even reversed by the choice of suspending fluid. He found that sodium chloride, potassium chloride, and potassium sulfate increased viability at intermediate RH

and decreased recovery at high RH, but did not affect it at low RH.

The effect of protecting agents and their mode of action on airborne microorganisms has been discussed by Cox (26) in terms of rates of water evaporation, and formation of viscous layers. The survival of airborne bacteria was found to be a function of the type of solute present in the spray fluid.

Effect of composition of the media

Various additives have been included in the aerosol collecting liquid media to improve efficiency of recovery.

Henderson (85) added alginate to the liquid impinger to prevent losses of bacteria during sampling.

Cox (27) found that bacteria (*E. coli* and *S. marcescens*) were unstable at a range of high RH when collected in phosphate buffer and the addition of raffinose to the spray fluid and the addition of M-sucrose to the collecting fluid increased the stability of *E. coli* commune and the recovery of *E. coli* strains B and Jepp.

Later (28) this difference in survival of E. coli commune was found to be due to the use of a variant organism.

The presence of sucrose in collecting fluid increased the survival for storage above 50% RH, but decreased it below 50% RH (29). Webb (149) reported no significant effect of the impinger fluid either on cells generated from distilled water or cultures sprayed with protective agents. Carboxymethylcellulose ether medium was shown (96) to be suitable for growing the samplings of cold aerosols.

Effect of atmospheric composition

The first attempts to control atmospheric conditions were directed toward air disinfection (65). A great variety of bactericidal agents for airborne bacteria were discussed by Sykes (134). These agents include epoxides, glycols, halogens, lactones, phenols, hypochlorites, and ozone. Attempts have been made to evaluate the effects of the positive and negative ions on airborne bacteria (94, 117).

Generally, experimental aerosols are generated in air which is assumed to be free from toxic components. Usually the air is only mechanically filtered (3) but charcoal and silica gel purification of the air has been reported (151).

Comparison of the effect of an inert atmosphere vs. air upon bacteria yielded similar results (142, 151) with E. coli and S. marcescens. Rosebury (122) indicated that an inert atmosphere did not improve the survival of Pasteurella tularensis at low RH. Lack of control of the physical loss of aerosol may have been the cause of lower recovery. However, many other investigators found that inert atmospheres increase the survival of aerosolized bacteria.

The replacement of air by nitrogen (59) increased the survival of E. coli, Micrococcus candidus, and S. marcescens. During the aerosolization of B. subtilis (var. niger) spores, Levin and Cabelli (97) noticed that the presence of oxygen affected the germination of spores. The survival of S. marcescens and E. coli at low RH levels was found to be dependent on the partial pressure of oxygen. Cox (27) found that three strains of E. coli survived better in nitrogen at low but not at high RH levels. Benbough's (14) findings were in agreement.

Cox (30) has also found similar results with argon and helium.

The presence of oxygen in air appears to affect microbial survival considerably. Therefore, experiments measuring the toxicity of oxygen have been conducted. Rogers (121) was one of the first investigators who recognized the toxic effects of oxygen on lyophilized cultures. Naylor and Smith (116) made similar observation on S. marcescens. These investigators found that organisms stored under vacuum survived the best, and had the lowest survival in oxygen atmosphere, whereas nitrogen, hydrogen, or carbon dioxide atmospheres produced intermediate results. The nature of the suspending medium before drying (126) was shown to influence the recovery of viable dried bacteria.

Lion (98) suggested that a prerequisite for effective protection against oxygen in the dry state is the accumulation of the solute around the bacteria. Benedict et al. (15) reported that atmospheric oxygen kills 95% of dried S. marcescens in 10 min, certain reducing agents prevent the action of the oxygen, and RH seems to play no role in the phenomenon. Wagman and Waneck (139) have

shown that the survival of water washed S. marcescens and E. coli depends on the residual moisture.

Studies of Zentner (164) concerning the effects of ascorbic acid on aerosolized S. marcescens suggest that interaction between the cells and atmospheric oxygen may contribute to death of cells. Hess (86) tested the effects of oxygen on aerosolized S. marcescens. He observed that the colony-forming ability of aerosolized organisms was rapidly destroyed by contact with 0.25% or more oxygen at 40% RH and 25 C, but was almost unimpaired in nitrogen (with not more than 10 ppm of oxygen) for 5 hr at 97% RH. The dehydration of cells seems to sensitize them to the lethal effects of oxygen. In freeze-dried microorganisms the formation of free radicals as the result of oxygen interaction has been proposed (48, 99) as a cause of death. An analogous lethal mechanism may operate in the aerosol.

The better survival of bacterial aerosols in an inert atmosphere than in air and the fact that death rates increase with oxygen content (27, 28, 29, 30, 34, 35, 86) strongly indicate the toxicity of oxygen on airborne microorganisms. The toxic effects of oxygen on the

colony-forming ability of E. coli were noted at low RH (34) whereby the T7 phage-replicating ability of the organism was retained.

Effect of radiation on airborne microorganisms

Ultraviolet (UV) rays are conveniently used for air disinfections. Monaci (112) observed that the bacterial population can be reduced by 20-99.9% depending upon experimental conditions. He found E. coli was the most sensitive, B. subtilis most resistant while S. aureus was intermediate. The effect of radiation is dependent on the amount, type (wavelength) and the specific organisms involved (156). There are no reports of the effects of particulate radiation on the aerosol (3). Beebe and Pirsch's experiments (13) showed that artificial sunlight linearly increased the decay rate of P. tularensis aerosols. The decay rate due to irradiation decreased linearly with increased RH. Radiation of Pasteurella pestis aerosols did not show linearity with light intensity. Increase in RH produced a linear protective effect between 25 to 80% RH but not above. Relative

humidity above 70% was found (68) to cause significant protection against natural sunlight. S. marcescens survived well (153) in artificial sunlight in air above 70% RH but it was less stable below this RH. The maximum rate of change took place between 65 and 55% RH for ultraviolet light. Inositol prevented radiation damage at these levels of RH.

The bactericidal action of solar range UV light (12) appeared to be proportional to the intensity of light. Moisture afforded varying degrees of protection against radiation. Webb (145) subjected airborne S. marcescens to prolonged exposure to 3400-4500 A and 5200-5800 A radiations. He found that besides these wavebands being lethal, they can also photo-reactivate cells previously irradiated with UV light of 2800-3200 A. Red alizarin dyes were protective against UV irradiation damage but blue and yellow dyes sensitized the cells. Ultraviolet and visible light produced an additive lethal effect when they were employed simultaneously. The effect of RH was studied on irradiated aerosols (147) and mutation of S. marcescens (147) and of E. coli (150) was observed. Spontaneous reactivation in dried cell aerosols of S. marcescens was reported to occur (46) after

short time exposure to UV radiation. Beebe (12) demonstrated that dry-disseminated aerosols were more resistant to the action of UV light at low RH levels than wet-generated aerosols of P. tularensis. Goodlow and Leonard (68) confirmed these findings.

Webb and Dumasia (154) reported that maximal X-ray damage occurred at 70-80% RH. At levels below 70% RH a sharp decrease in the sensitivity of aerosolized E. coli to the radiation occurred (i.e. the decrease in viability by other factors such as removal of water by evaporation is so great that X-rays do not contribute more apparent damage to the cells). These authors suggested that the removal by ionization of a strategic bound water molecule by X-rays cause most of the cell deaths.

Webb (148) showed that the inactivation of airborne Rous sarcoma virus by UV light depended on RH. A very rapid increase in the sensitivity of the virus to UV light was observed as the RH was lowered from 70 to 50%. Inositol protected Rous sarcoma virus exposed to UV light. Simultaneous UV light irradiation with spraying strains of Mycoplasma (92) destroyed more than 99% of the

population which did not decrease by one log cycle during 45 min exposure to 23% RH.

Basic causes of death of aerosolized microorganisms

In aerosol experiments the inability of bacteria to form colonies or the inability of phages and viruses to replicate themselves is taken as proof of the loss of viability. Damage to the organisms can occur during the different stages of aerosolization: 1) while the aerosol is being generated; 2) when the organisms become airborne; and 3) during sampling of aerosols. However, the cause of death may become indistinguishable as a result of interaction among many factors.

The most extensively studied aspect of loss of viability by airborne bacteria is the relationship between water content of bacteria, relative humidity of air, and their effect on cellular structures. Although in other studies the approach might be different, the indications are such that an intimate relationship between loss of viability and dehydration does exist.

Brown (20) suggested the possibility of two different death mechanisms operating at high and low relative humidities in airborne E. coli. The mechanism at low RH was attributed to the water activity effect.

Webb (142) suggested that movements of water molecules in and out of the cell resulted in a collapse of the natural structure of cellular protein. He has correlated death rates of airborne cells and temperature and RH with a mathematical function. He found that activation energies associated with aerosol death increased with aerosol age. Later, he proposed (144) that the site of damage is the cellular membranes which break down on desiccation. In turn, this breakdown of tertiary protein structure brings about a loss of differentiation within the cell. In a subsequent paper (146) he changed this view by suggesting that a structural change in the nucleoproteins concerned with protein synthesis is responsible for the death of dried cells of E. coli.

While investigating the effects of desiccation on metabolic systems in E. coli, Webb (146) observed that dehydration increased the release of 260 nm absorbing material from the cell. On the basis of this finding and that inositol offered protection against desiccation,

he suggested that a change in the nucleoprotein structure was responsible for cell death. Webb et al. (152) showed that Rous sarcoma virus was protected by inositol. They suggested that the 99.9% of airborne organisms die as a result of an irreversible change in the structure of nucleic acids due to the loss of water molecules.

Webb (150) reported that aerosolization and irradiation of E. coli produced mutants of this organism. He suggested that mutation is one step towards death. Thus mutation or a change in DNA usually results in progenies of limited viability under certain conditions. Webb (151) stated that the death rate of airborne cells is directly related to the amount of bound water removed, and, since there is an apparent damage to DNA, the desiccation of the cells is mutagenic. Webb et al. (155) proposed the hypothesis that desiccation damages the cell and viral DNA through removal or reorientation of the bound water.

Hayakawa and Poon (82) related the mechanism of instantaneous killing of airborne bacteria to the evaporation of cellular water. Webb's (151) thermodynamical analysis of cell death rates indicated that a tightening of molecular structures occurred during desiccation. He proposed that as a result of loss of water from airborne

microorganisms, interaction between oxidizing and reducing groups of cell structures took place. Death could be prevented by keeping these groups apart by substituting inositol for water in its hydrogen bonding capacity. He proposed that enzymes were not affected by drying, therefore, they could not be blamed for loss of viability of airborne organisms.

The resistance of enzymes to aerosolization was used by Anderson (2) as a means of the determination of total populations in aerosols. He also demonstrated that the protein synthesizing ability of E. coli was damaged even in the first few seconds of airborne state. He suggested that the study of protein synthesis in aerosolized cells immediately after recovery might yield valuable information on the site of the lethal process, and there might be at least two sequential lethal processes in airborne E. coli.

Cox and Baldwin (34) found that the DNA of T7 phage was affected when phage infected E. coli were aerosolized. They demonstrated that the mechanisms of death at high RH were different from death caused by oxygen. Benbough (14) described two possible mechanisms which may contribute to death of airborne E. coli: 1) at low

RH the action of oxygen caused damage to flavin-linked enzymes as a result of free radical activity, hence the protective effect of free radical suppressors, and electron transport inhibitors against the lethal effects of oxygen; 2) death at high RH resulted from the effect of aerosolization on RNA synthesis; it was suggested this caused some lethal biosynthesis of proteins which can be prevented by action of RNAse on the critical s-RNA fraction.

Cox (31) ascribed the cause of death to failure of RNA synthesis, protein synthesis or energy production. In a subsequent paper (32) he stated that rehydration caused dissociation of the RNA complex and subsequent hydrolysis. He found higher aerosol survival at 40% RH than at 80% difficult to explain on the basis of rehydration damage.

A toxic effect of solute concentration on microorganisms as a result of loss of water has been suggested by several investigators (10, 53, 114). Crystallization of solutes, as a result of water loss, was reported to affect viability of microorganisms (129).

Death of airborne microorganisms may occur during collection. Anderson and Cox (3) stated that a transient osmotic shock, sufficient to break cell membrane, may cause death during collection as a result of rehydration. To avoid this effect, fluids of high osmotic pressure have been used for the collection of airborne microorganisms. Since M-sucrose plus phosphate buffer in the collection liquid was shown to protect E. coli when sprayed into relatively high humidities (28) it was possible that the protective action of solutes during collection was not affected by equilibrating the osmotic pressure (26, 27, 28, 34). Anderson and Cox stated (3) that the extent of osmotic damage depends on the robustness of the microbes. Thus P. tularensis is more sensitive than E. coli. Aged cells might be expected to have a greater resistance than young organisms (3).

Ferry et al. (60) ascribed the loss of viability in aerosolized E. coli mainly to physical stresses of aerosol generation and collection. May and Harper (107) have stated that sonic velocity in impingement was shown to have a lethal effect on the more sensitive type of bacterial cells. The damage can be reduced by the multiple orifice impinger designed by George et al. (66),

and by reducing impingement velocity (107). Prolonged collection of aerosols, both by liquid impingement (66) and solid agar surface impaction (95) was found to decrease the survival.

In the metabolic activities of aerosolized organisms' protein synthesis, phage replication, and the control of ion balance was studied (5, 6, 32, 33). Drying of E. coli on membrane filters was shown (155) to decrease the ability of the organism to synthesize β-galactosidase. Anderson (2) found that the loss of ability of E. coli to synthesize β-galactosidase preceded death in aerosol. Webb et al. (155) found that aerosolization of E. coli B decreased its capacity to replicate T1, T3, and T7 phages over the range of 30-80% RH, while the replication capacity for T2 and T4 phages was unaffected. They showed that loss of ability to form colonies by aerosolized E. coli preceded its loss of ability to replicate odd numbered phages. Webb et al. (155) did not detect appreciable impairment to colony-forming or phage-replicating ability of E. coli at high RH levels (70-80% RH).

Experiments of Cox and Baldwin (34) showed that E. coli B and its T7 phage were affected by the same lethal processes at high RH, between 70-100%. Different death

mechanisms were responsible at low RH in the two organisms. At low RH levels oxygen was involved in the death mechanism in aerosolized E. coli B (35). The death mechanism caused by oxygen was shown to operate differently from those which occurred at high RH (14, 27, 28, 29, 34).

Studies by Anderson and Dark (5) on ^{43}K labeled E. coli indicated that this radioisotope was lost very shortly after recovery from aerosols, except at high RH, and short holding times. The loss of potassium did not appear to be due to the physical stresses of aerosol generation and collection. The loss was merely a sequel to aerosolization but not a direct cause of death. These effects have been confirmed in various other microorganisms by Anderson et al. (6).

Maltman (103) demonstrated that desiccation and rehydration during aerosolization of staphylococci produced leakage of amino acids, phosphates, protein and ribonucleic acid components from the cell. His experiments indicated that the initial rate of protein synthesis was slower in the cells which survived the desiccation and rehydration process. This was attributed to the deleterious effect of desiccation on the synthesis of RNA. The rate of RNA synthesis returned to normal values

more quickly in log phase cells than in stationary phase organisms although stationary phase cells are considered more resistant to these stresses than young organisms.

METHODS AND MATERIALS

Determination of viability of airborne *Salmonella newbrunswick*

Figure 1 gives a diagrammatic representation of the equipment used for studying the survival of airborne *S. newbrunswick* originally isolated from non-fat dry milk (by the FDA Laboratory in Detroit, Michigan). The bacteria were grown in nutrient broth on a rotary shaker for 20 hr. The culture was centrifuged for 3 min at 12,100 x g (Sorvall SS1 centrifuge); the pellets were washed three times in 0.25 M phosphate buffer and resuspended in distilled water (or skim milk).

These suspensions were aerosolized by a De Vilbiss 40 atomizer operated at 20 psig with compressed air. The aerosol was passed through a 1 ft^3 prechamber to stabilize droplet size and then into a sealed stainless steel chamber with an inner space of 5' x 4 x 5' (100 ft^3). The aerosol in the chamber was circulated by an electric fan operated at 790 ft^3/min during the aerosolization. The temperature and the RH of the chamber was controlled at

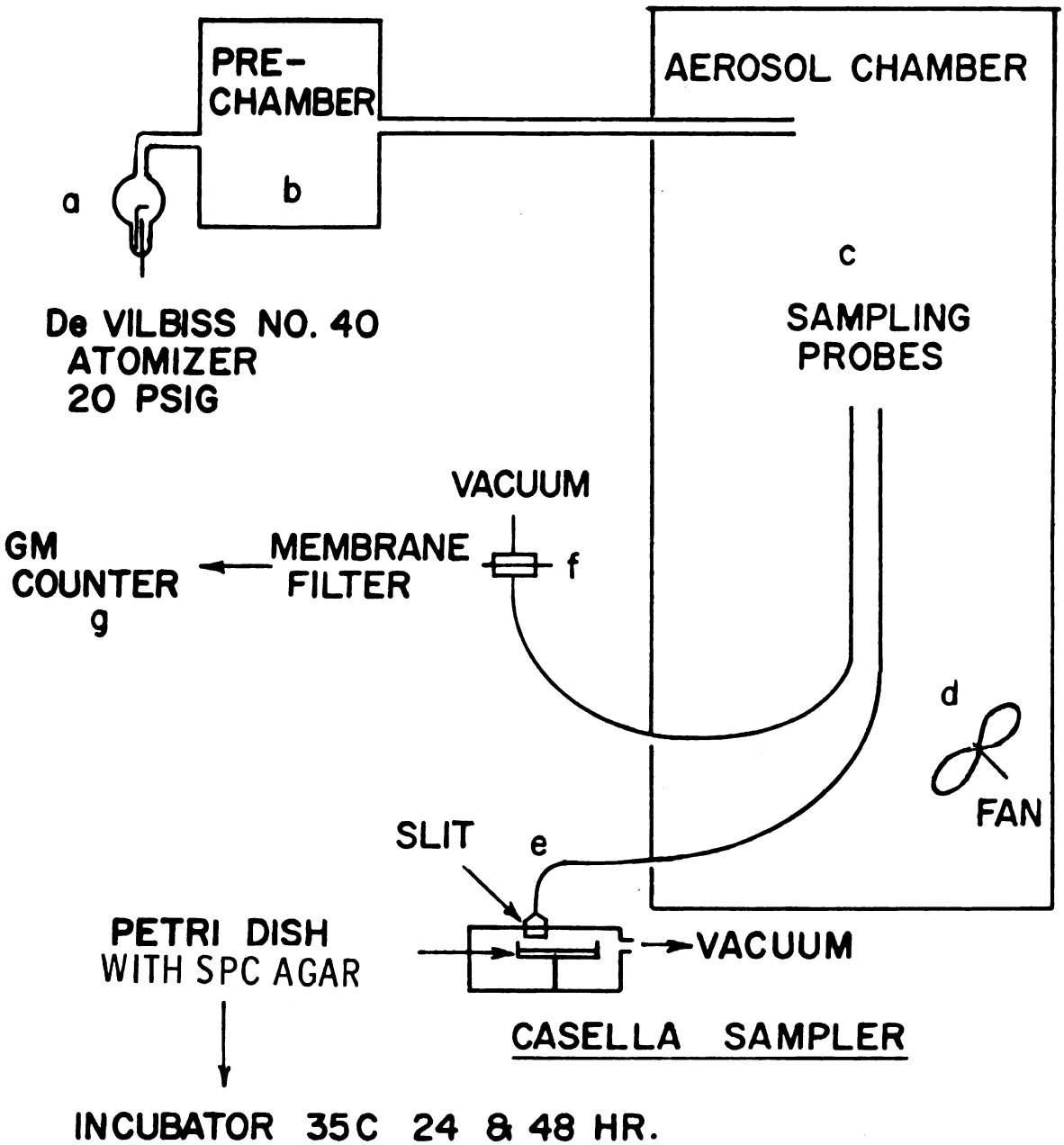

Figure 1 Equipment for studying the survival and sampling of airborne Salmonella newbrunswick.

10 or 21 C and at 30, 50, 70 or 90% RH. Constant temperature was maintained with heating and cooling equipment above the false ceiling. The RH was controlled by a spray, exposed water, and/or silica gel. Temperature and RH were continuously recorded by a Honeywell 24 hr recorder.

Separate batches of S. newbrunswick were grown in 20 ml of low phosphate medium in the presence of radioactive dipotassium phosphate ($K_2H^{32}PO_4$) with 0.5 mc activity per ml of medium. The labeled cells were killed by holding them at 80 C for 15 min. The radioactive phosphorous cells were washed to remove the free $K_2H^{32}PO_4$ with 0.01% "Triton X 100" until the supernatant fluid contained no radioactivity perceivable by the Geiger-Müller counter (Nuclear-Chicago). The labeled cells were dispensed into vials with water and freeze dried.

At the beginning of each experiment, appropriate dilutions of the freshly grown suspensions of S. newbrunswick were prepared with the radioactive phosphorous (^{32}P) dead cells. The suspension was well mixed in a Vortex Jr. Mixer and transferred to the De Vilbiss 40 atomizer. Air samples were taken after aerosolization of the suspensions for 5 min, and at 10 min intervals for 90 min. At each

time interval, a sample of 1 ft^3 of air was taken by the Casella air sampler using standard plate count agar. Growth of colonies indicated the number of viable cells per ft^3 of air.

Another sample of 0.5 ft^3 was taken simultaneously through a membrane filter (Millipore, Co. 0.45μm pore size, 25 mm diameter) to establish the total bacterial number by means of the ^{32}P in the cells. After sampling, the agar was overlayed with melted agar (held at 45 C). Plates were incubated at 35 C for 24 hr and the colonies counted. Further incubation for 48 hr usually did not result in additional colonies.

To establish the concentration of aerosol, the membrane filter was transferred from the filter holder to aluminum planchets and each was covered with thin plastic tape. Counting was by means of a Geiger-Müller counter.

Determination of efflux materials from airborne S. newbrunswick

Preparation of the airborne samples

S. newbrunswick was grown at 37 C in chemically defined media (32). The cells were harvested by

centrifuging (Sorvall SS 1) at 12,100 x g at room temperature, suspension in deionized sterile water, and subjected to three similar centrifugings. The final suspension was atomized into a temperature and RH equilibrated air stream produced by an Aminco unit (Figure 2) and drawn by a vacuum pump.

The airborne bacteria were collected after being airborne from the air stream and exposed to the air stream while on the membrane filters (Figure 2 A and B). The two filters with the collected bacteria were held in the vacuum produced air stream from the Aminco unit for one half hr. Then, one filter (Figure 2 A) was removed from the air stream and the microorganisms rehydrated rapidly by plunging the filter into distilled water.

The other filter (Figure 2 B) was exposed to an air stream of gradually increasing relative humidity to near 100%. The gradual increase of relative humidity was accomplished by raising the temperature of the water bath of the Aminco unit to the dry-bulb temperature of the air thus giving a 100% saturation at the operating temperature (80 F). At higher temperature and RH condensation occurred.

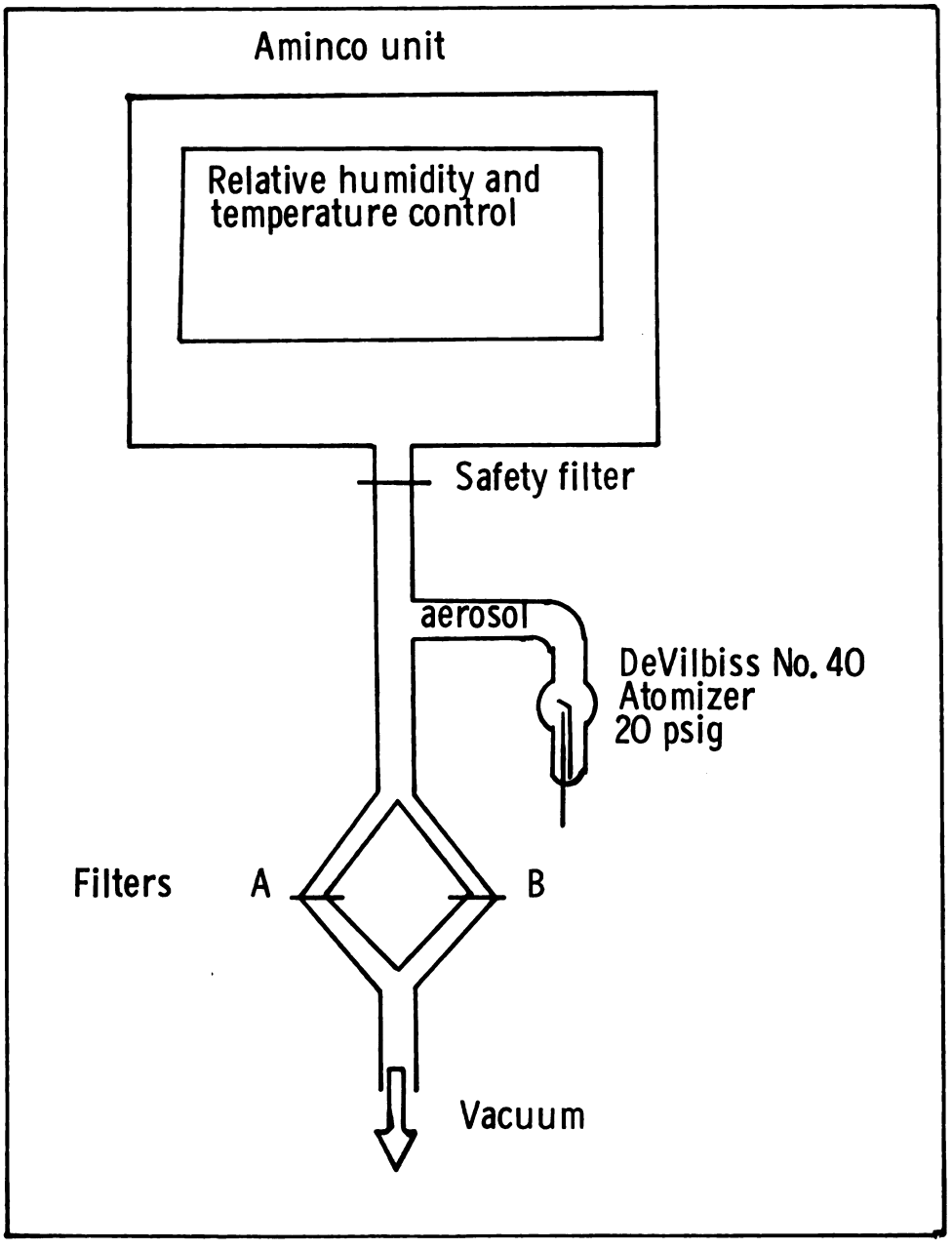

Figure 2. Equipment for aerosolizing, equilibrating and rehydrating airborne Salmonella newbrunswick.

When the RH of the air stream reached 95% or more the filters were transferred to a beaker with a few drops of water and rehydrated with the same quantity of water as the rapidly rehydrated sample. The cells were resuspended in the distilled water and the absorbance of the samples was determined at 600 nm by a Spectronic 20 (Bausch & Lomb) spectrophotometer. The absorbance of all samples were adjusted to a 0.3 value (corresponding to 4.6×10^8 cells/ml) by diluting the samples with the required amount of distilled water. This provided similar osmotic conditions for all the treatments: 1) control (unaerosolized cells), 2) rapidly rehydrated, and 3) gradually rehydrated cells. After the adjustment of the absorbance, the cell suspensions were incubated in distilled water at 35 C for 1/2 hr and then centrifuged.

The supernate of the cells of each treatment was passed through a 0.45μ pore size membrane filter into an acid-cleaned sterile test tube. Its absorbance was determined at 265 nm by the DBG Beckman Spectrophotometer. The remainder of the supernatant fluid was frozen at -40 C for subsequent chemical analyses. The cell pellets were freeze-dried for electron microscopy in the screw

cap tubes in which centrifugation took place. The pellets were freeze-dried in 2 hr. The vacuum in the tubes was replaced by nitrogen gas of 99.99+% purity.

Spot tests for inorganic ions

The presence of inorganic materials in the supernatant fluids of rehydrated cells of airborne S. newbrunswick was determined by Feigl's spot tests (56) with slight modifications.

Potassium (K) was determined by spotting the supernatant fluid of bacterial suspension on filter paper impregnated with sodium dipicrylaminate. The paper was dried in a current of heated air and placed in a petri dish with 0.1 N nitric acid. The presence of potassium was indicated by the appearance of red fleck or ring after the change of the original orange-red color of the paper to bright yellow. Limit of identification was $3X10^{-6}$ g potassium and the limit of dilution was 1:16,000.

Preparation of the reagents was as follows: 0.2 g dipicrylamine was dissolved in 2 ml of 2 N sodium carbonate and 15 ml of deionized water. Strips of paper were soaked in the solution and dried in hot air.

Sodium (Na) was determined by placing a drop of the supernatant fluid on a dark spot plate. It was mixed with 8 drops of the reagent solution. The formation of yellow turbidity or green-yellow fluorescent precipitate under the ultraviolet lamp was taken as an indication of the presence of sodium. Limit of identification was 2.5×10^{-6} g of sodium. Limit of dilution was 1:20,000.

Preparation of the reagents for sodium was as follows: solution of zinc uranyl acetate: a) 10 g of uranylacetate was dissolved by warming in 6 g of 30% acetic acid, and diluting with water to 50 ml. b) 30 g zinc acetate was stirred with 3 g 30% acetic acid, and diluted with water to 50 ml. Warm suspensions of (a) and (b) were mixed to form a solution. A trace of sodium chloride was added after 24 hr; the slight precipitate of sodium zinc uranyl acetate was filtered and discarded.

Phosphate (PO_4) was determined by placing a drop of the supernatant fluid on filter paper followed by a drop of molybdate and a drop of benzidine solution. Then the paper was held over ammonia. The appearance of blue stain indicated the presence of phosphate. Limit of

dilution was 1:4,000 and the limit of identification was $1.2X10^{-6}$ g.

Preparation of the reagents for phosphates was as follows: 1) ammonium molybdate solution: 5 g salt was dissolved in 100 ml cold deionized water and poured into 35 ml nitric acid (Sp gr 1.2); 2) benzidine solution: 0.05 g benzidine was dissolved in 10 ml concentrated acetic acid and diluted with deionized water to 100 ml.

Magnesium (Mg) was determined by placing a drop of the supernatant fluid and a drop of deionized water in adjoining depressions of a white spot plate and mixing with 2 drops of alcoholic 0.02% solution of quinalizarin; 2 N sodium hydroxide was added drop by drop until the yellow-red color of the solution changed to blue-violet, then an excess of 1/4 to 1/2 of the volume present was added. The appearance of blue precipitate or coloration indicated the presence of magnesium. The blank remained blue-violet. The difference in shade was intensified by holding while the dyestuff decomposed in the magnesium free solution. The colored magnesium compound is stable. Limit of identification was 0.25×10^{-6} g magnesium and the limit of dilution was 1:200,000.

Spot tests for organic compounds

Organic compounds in the supernates of rehydrated airborne S. newbrunswick were determined by slightly modified spot tests of Feigl (57).

Simple carbohydrates (with free aldehyde group) were determined by oxidation with periodate. A drop of the supernatant fluid was placed on a white spot plate with a drop of 5% potassium periodate solution and a drop of 10% sulfuric acid (V/V) and the mixture was allowed to stand for 5 min. The excess periodic acid was reduced with a few drops of saturated sulfurous acid, and the sample was treated with a drop of fuchsin-sulfurous acid. The appearance of red indicated the presence of simple carbohydrates. Limits of detection were 1×10^{-6} g formaldehyde, 4×10^{-6} g acetaldehyde, 5×10^{-6} g glycol, 2.5×10^{-6} g glycerol, 5×10^{-6} g mannitol, 25×10^{-6} g fructose, 25×10^{-6} g lactose, 25×10^{-6} g arabinose.

Preparation of the fuchsin-sulfurous acid reagent for simple carbohydrates was as follows: sulfur dioxide was passed through a 0.1% aqueous solution of fuchsin until the color disappeared.

Amino acids were detected by testing for the presence of free amines. One drop of the supernatant fluid was evaporated to dryness in a micro test tube, and held for a short time at 100 C. An excess of potassium thiocyanate dried to constant weight was added and the mixture was heated in a bath to about 200 to 250 C. A filter paper was moistened with a 10% solution of lead acetate and it was placed over the mouth of the test tube. The presence of amino acids was indicated by the appearance of a black stain on the paper over the test tube. The following amino acids give positive reaction: glycine, phenylalanine, tyrosine, aspartic acid, methionine, and leucine.

Proteins were detected with tetrabromophenolphthalein ethyl ester. A drop of the supernatant fluid was mixed on a white spot plate with a drop of the blue reagent solution and then acidified with a drop of 0.2 N acetic acid. The blank turned yellow but the blue to greenish color persisted in the sample containing protein. Limit of identification was 10^{-6} g for various proteins. The reagent for protein detection was 0.1% solution of the potassium salt of tetrabromophenolphthalein ethyl ester in alcohol.

The presence of nucleotides was detected by measuring the absorbance of the supernate at 265 nm with a DBG Beckman spectrophotometer against a distilled water blank.

Separation of nucleotides was carried out by column chromotography on dextran Biogel 2 which permitted the separation of molecules ranging between molecular weights of about 150 to 2,500. The elution was carried out by 7 M urea (88) in 0.02 M Tris/HCl buffer at pH 7.6 (42). The void volume of the column was determined by blue dextran in 7 M urea and the Tris buffer. The position of a peak for mono-nucleotides was established by using yeast adenosine monophosphate eluted on the same column.

Electron microscopy of airborne *Salmonella newbrunswick*

Fixation of aerosolized *S. newbrunswick* cells for electron microscopy was performed by a method of Stoeckenius and Rowen (131) with slight modifications. The cells preserved as freeze-dried pellets were fixed with 4% formaldehyde in a stock salt solution (1% NaCl and

0.2% $CaCl_2$) at 4 C for 6-7 hr. The cells were then centrifuged at 3,020 x g on Sorvall SS 1 centrifuge. The pellet was resuspended in the salt solution and held overnight at 4 C. After centrifugation and the removal of the supernatant fluid, a drop of 1.5% melted agar in the salt solution was added to the pellet and cooled to 4 C during 1/2 hr. Postfixation of the agar pre-embedded pellet was accomplished in 1% osmium tetroxide in the stock salt solution for 6 hr at 4 C. The agar block was washed in deionized water for 30 min at room temperature. The agar embedded pellet was broken up to small pieces and stained with 2% aqueous uranyl nitrate for 30 min at room temperature. The agar blocks were dehydrated in graded acetone according to method by Reyter and Kellenberger (120). The dehydrated agar blocks were embedded in Epon for sectioning. The Epon was prepared by mixing 5 ml of Epon A (Appendix 1) and 5 ml of Epon B (Appendix 1) with the addition of 4 drops of DMP-30 accelerator.

The Epon mixture was added to an equal quantity of 100% acetone by volume and the dehydrated agar blocks were held in this mixture overnight at room temperature.

The 1:1 Epon: acetone mixture was replaced by 100% Epon for 12 hr and followed by another change of 100% Epon for 12 hr. Then, the Epon-impregnated agar blocks were transferred to the final 100% Epon in gelatin capsules. The Epon was hardened for 48 hr at 60 C and a week at room temperature. The sectioning was carried out by Sorvall MT 2 microtome using freshly broken glass knives. Only silver sections were used. The thickness was estimated to be about 50-60 nm. Electron micrographs were obtained with the Philips EM-100 Electron Microscope.

Determination of the effect of a bipolar-oriented electrical field on airborne microorganisms

In this study two identical stainless steel chambers described previously were used with a special plastic coated electrode[a] (4 X 4 X 32 in) for producing the bipolar-oriented electrical field. The electrode was suspended from ceiling to within 2 ft of the floor in each chamber. Various voltages were applied to the electrode in the test chamber whereas the electrode in the control chamber was not charged.

[a]Invented by C. D. Slocum, Sr. and supplied by Envitron Corporation, Royal Oak, Michigan 48067.

The microbial species used in the trials included: a) Serratia marcescens grown in nutrient broth (shake culture) at 32 C, b) Pseudomonas fragi similarly grown at 28 C, c) Bacillus subtilis spores in Roux flasks with nutrient agar at 28 C, d) Candida lipolytica grown in Sabouraud maltose broth at 28 C (shake culture), and e) Penicillium roqueforti obtained as Blue Cheese Mold Powder from a commercial source. Each species was washed four times in 0.25 M phosphate buffer and dispersed in distilled water according to the desired dilution for aerosolization.

During the test period of 5 to 8 hr a species was continuously aerosolized into the test chamber and the control chamber from the 1 ft^3 prechamber through two plastic tubes of 3/8 in ID.

In a few trials, when the effect of the bipolar-oriented electrical field on viability of S. marcescens was to be determined, the aerosolization was for 5 min at the beginning of the test period.

The airborne microorganisms were sampled by the Casella sampler using petri plates with standard plate count agar. For the yeast or mold, acidified potato

dextrose agar was used in the air sampler. Each sampling consisted of 1 ft^3 of air. Rodac plates were used for determining the numbers of organisms on the wall, door, floor, and electrode by direct agar contact. The air sampling was at 1/2 hr intervals from 5 to 8 hr.

RESULTS AND DISCUSSION

Viability of airborne Salmonella newbrunswick

The survival of airborne S. newbrunswick was determined by aerosolization from distilled water or skim milk in order to reveal the organism's natural resistance, or the degree of protection provided by organic substances such as skim milk solids.

The resistance data was used to determine activation energies and entropies related to death of airborne S. newbrunswick.

Evaluation of the biological decay

The survival (%S) curve for airborne S. newbrunswick (Figure 3) was plotted on the basis of the percentage of total viable count* (%T) and total population (%P) by

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*Total viable count of airborne bacteria as used here is the decreasing viable count on the agar plates, the decrease being due to two factors: 1) biological, i.e.

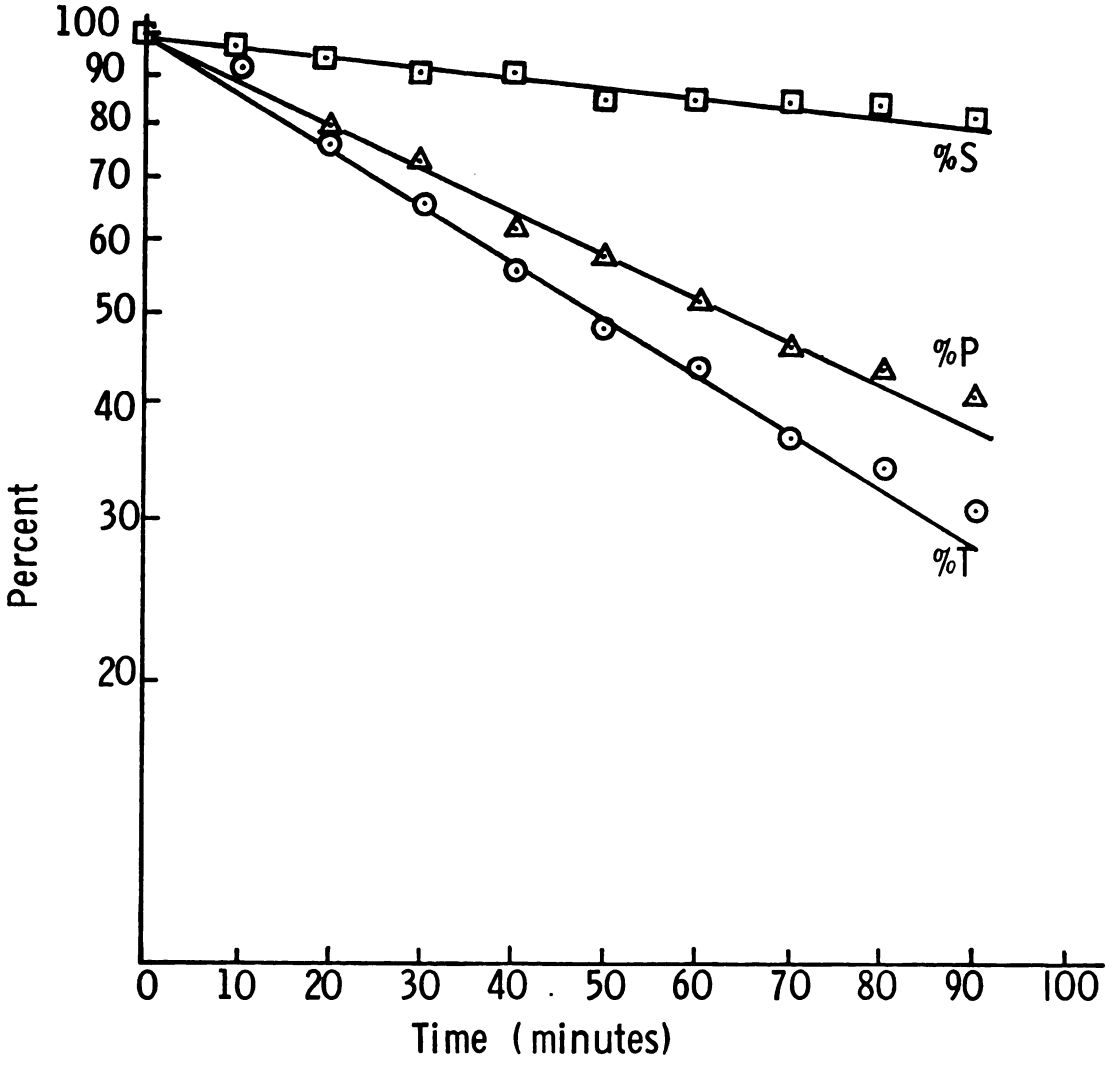

Figure 3. Survival (%S), total population (%P), and viable population (%T) curves of airborne Salmonella newbrunswick aerosolized from skim milk at 10 C and 30 % RH.

using the formula $\%S = \frac{\%T}{\%P} \times 100$. The natural logarithms ($\log_e$) of the slopes of the best fit survival curves were taken to obtain the death rates (K values) omitting their negative sign. These K values were also converted into decimal reduction times so that they might be related to thermal resistance data available in other areas of food science.

Formula for the relationship between K and D values:

$$KD = \log_e N_1 - \log_e N_2$$

where: N_1 = percentage of organisms at 0 time

N_2 = organisms reduced by 90%

K = natural logarithm of the slope of the survival curve

D = time in min necessary to reduce the population of bacteria by 90%.

Depending on experimental conditions, straight or broken survival curves were obtained as shown by Figure 4. Accordingly, single (K_S), initial (K_I) for the first 20 min,

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dying of bacteria in the air and 2) the physical removal of bacteria from air due to settling, attraction to the walls, etc.

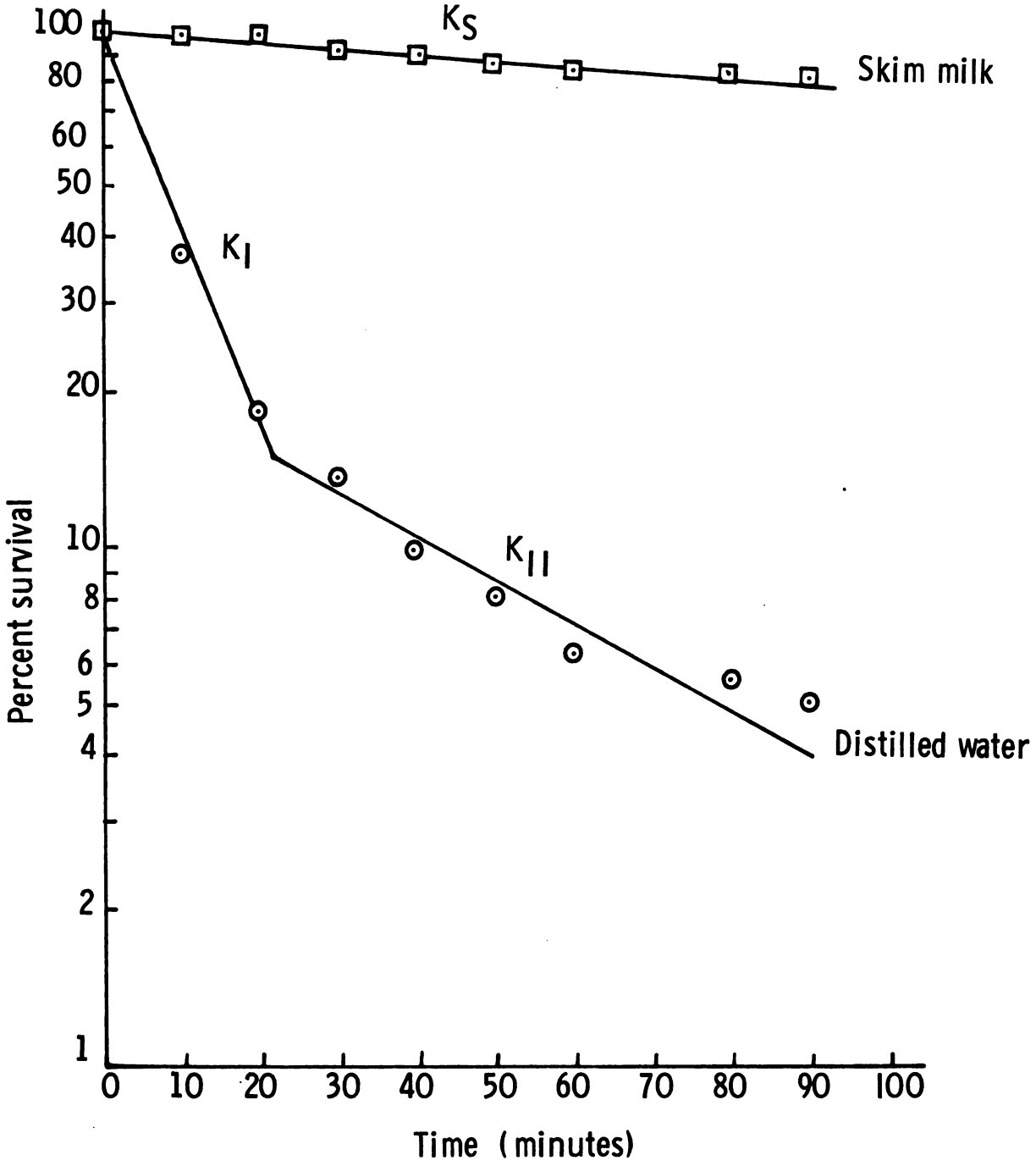

Figure 4. Survival curves of airborne Salmonella newbrunswick aerosolized from skim milk or distilled water at 10 C and 30% RH. (K_I) initial death rate; (K_{II}) secondary death rate ; (K_S) single death rate.

and secondary (K_{II}) during 20-90 min death rates were calculated.

The effect of relative humidity, temperature, and suspending medium

Table 1 (five or six trials for every condition) shows the viability of S. newbrunswick at 10 and 21 C and at 30, 50, 70, and 90% RH expressed both as K values and as decimal reduction times, or D values.

At 21 C and 90% RH the death rate of airborne S. newbrunswick appeared to be the same throughout the 90 min period when the organism was aerosolized from distilled water (K_I and K_{II} = 0.0141). The S. newbrunswick suspended in skim milk showed a similar death rate to distilled water aerosolized bacteria (K_S = 0.0140) under the same conditions. Of particular interest is that the D values corresponding to these death rates were 163 and 164 min, resp.

If the RH was decreased to 70% at 21 C an initial higher death rate (K_I = 0.0288) was observed when the organism was sprayed with distilled water. The death rate decreased during the following period (K_{II} = 0.0175).

Table 1.--Death rates (K) and decimal reduction times (D) of Salmonella newbrunswick at 10 and 21 C and at four RH levels.

% RH		Aerosolizing medium					
		Distilled water				Skim milk	
		K_I		K_{II}		K_S	
		10 C	21 C	10 C	21 C	10 C	21 C
30	K	0.0346	0.0560	0.0099	0.0182	0.0057	0.0049
	D	67	41	233	126	404	470
50	K	0.0330	0.0590	0.0112	0.0213	0.0077	0.0085
	D	70	39	206	108	299	271
70	K	0.0191	0.0288	0.0085	0.0175	0.0075	0.0114
	D	121	80	271	132	307	202
90	K	0.0112	0.0141	0.0057	0.0141	0.0094	0.0140
	D	206	163	404	163	245	164

Further decrease in RH to 50 and 30% increased the initial death rates (K_I = 0.059 and K_I = 0.0056, resp.). The secondary death rates were also increased, especially at 50% RH (K_{II} = 0.0213), but not as much at 30% (K_{II} = 0.0182).

These death rates above are similar to those obtained by Webb (151) using E. coli and S. marcescens. He also observed a decrease of death rate with time and found that K values for E. coli and S. marcescens during the 0-1 hr observation period at 30% RH and 23 C were 0.031 and 0.036. These values decreased during 1-5 hr aerosol storage periods to 0.025 and 0.022, resp. Initially more rapid death rates were also observed for airborne E. coli by other investigators (58, 82). Data of Ehrlich et al. (54) on the survival of S. marcescens indicates sharp decrease in slope of survival curves, the change being apparent at 16 or 32 min depending on temperature.

When S. newbrunswick was suspended in skim milk and aerosolized, no distinct secondary death rates could be observed (e.g. Figure 4). The death rate at 21 C decreased with a decrease in RH (from K_S = 0.0140 at 90% RH to K_S = 0.0049 at 30% RH). At 30% RH the corresponding decimal reduction time was 470 min signifying that 10% of the airborne S. newbrunswick might survive for a similar length of time.

These results agree in general with those of Webb (151). He noticed similar effects of added organic chemicals to the atomizing medium on the death rates of

airborne E. coli and S. marcescens. Webb also found that partial hydrolyzates of casein and hemoglobin had decreased considerably the death rates of E. coli and S. marcescens during the 0-1 hr and 1-5 hr aerosol age. Death rates decreased with change of RH from 30% to 50% when certain organic compounds were added to the atomizing media. However, addition of some organic compounds had a reverse effect.

The addition of inositol, mono-, di-, and trisaccharides was protective (151). Lactose was not investigated. It is conceivable, however, that with skim milk proteins, lactose and some soluble nitrogen compounds, amino acids, and peptides (amino groups) contribute to the protection of S. newbrunswick in air. Webb (151) claims that small chemical compounds such as polyhydroxy alcohols, and amino acids possessing hydroxyl, amino, and possibly sulfhydryl groups have protecting effects on bacteria by replacing bound water to maintain biological integrity of macromolecules.

Cox (27) theorized that protective agents may act by forming a highly viscous layer between the cell wall and its environment, hence, slowing the rate of access of

oxygen to the bacterium. Oxygen (14, 15) has been reported to cause damage to flavin-linked enzymes as the result of free radical activity. Cox (27) also claimed that the highly viscous protective substance may behave as a non-volatile solvent for structures in the cell wall which become labile to air only when desiccation occurs.

A similar trend of increasing death rate at decreasing RH and 21 C of airborne S. newbrunswick was also observed at 10 C (Table 1). The lowest initial death rate for a distilled water suspension of S. newbrunswick at 10 C was observed at 90% RH (K_I = 0.0112) among the RH levels tested which corresponds to a D_{10C} value of 206 min. This D_{10C} value was greater than the D_{21C} of 163 min. At 10 C the initial death rate of airborne S. newbrunswick aerosolized with distilled water increased as the RH was decreased to 70 and 50% (K_I = 0.0191, and K_I = 0.033, resp.). At 10 C and 90% RH the secondary death rate was K_{II} = 0.0057 and it increased as the RH was decreased to 70 and 50% (K_{II} = 0.0085 and 0.0112, resp.). Further decrease to 30% RH did not change the secondary death rate significantly (K_{II} = 0.0099).

When S. newbrunswick was aerosolized in a skim milk suspension at 10 C at the various RH levels, there

was no distinct break in the survival curve and, therefore, only one death rate was calculated. A lower death rate of S. newbrunswick aerosolized in skim milk was observed at 90% RH (K_S = 0.0094) than in distilled water under the same conditions (K_I = 0.0112) indicating the protective action of skim milk. The initial death rate of S. newbrunswick aerosolized from skim milk decreased as the RH was decreased from 90 (K_S = 0.0094) to 70 and 50% (K_S = 0.0075 and K_S = 0.0077, resp.). The lowest initial death rate corresponding to D value of 404 min was observed when the RH was decreased to 30% (K_S = 0.0057). The increase in resistance with the decrease in RH may be due to more rapid evaporation of water from the surface of the bacteria and subsequent formation of a protective layer of milk solids which reduces removal of water from within the cells, or protects cells from deleterious effects of oxygen (14, 15).

Special significance may be ascribed to these observations under food manufacturing conditions. When these organisms become airborne from drains or other sources in a food processing plant (85) where organic protective materials are usually available, they may have ample opportunity to travel and survive to contaminate

food products. Under favorable conditions survival and propagation of salmonellae might result.

Figure 5 shows that the initial death rates moderately increased at all RH levels when the temperature was increased from 10 to 21 C.

The moderate increase in the death rates as a result of increasing temperature from 10 to 21 C especially at the higher RH levels, may indicate that the death rates at 10 or 21 C are more dependent on the water vapor content of the air than on changes in temperature. This relative temperature independence of death rates is shown by data of Ehrlich et al. (54). They found that viability of S. marcescens did not change considerably from -18 to +24 C. A rapid increase in death rate was observed for S. marcescens and E. coli above 24 C. This phenomenon might be related to changing vapor pressure of water because between 0 and 25 C there is a moderate change of water vapor pressure with increase in temperature. However, beyond this point the water vapor increases exponentially (Figure 6, based on the water vapor pressure table in Handbook of Chemistry and Physics, 74).

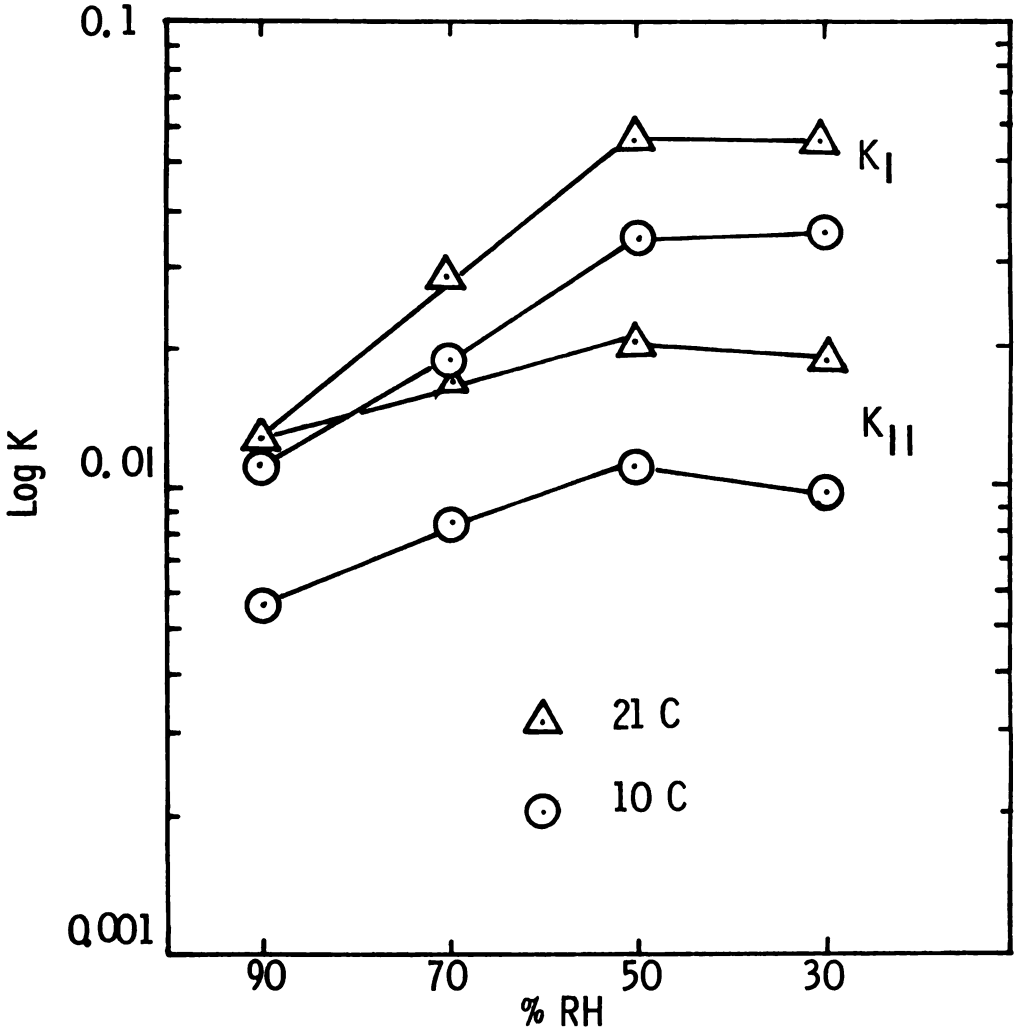

Figure 5. Initial (K_I) and secondary (K_{II}) death rates of Salmonella newbrunswick aerosolized from distilled water at four RH levels and 10 or 21 C.

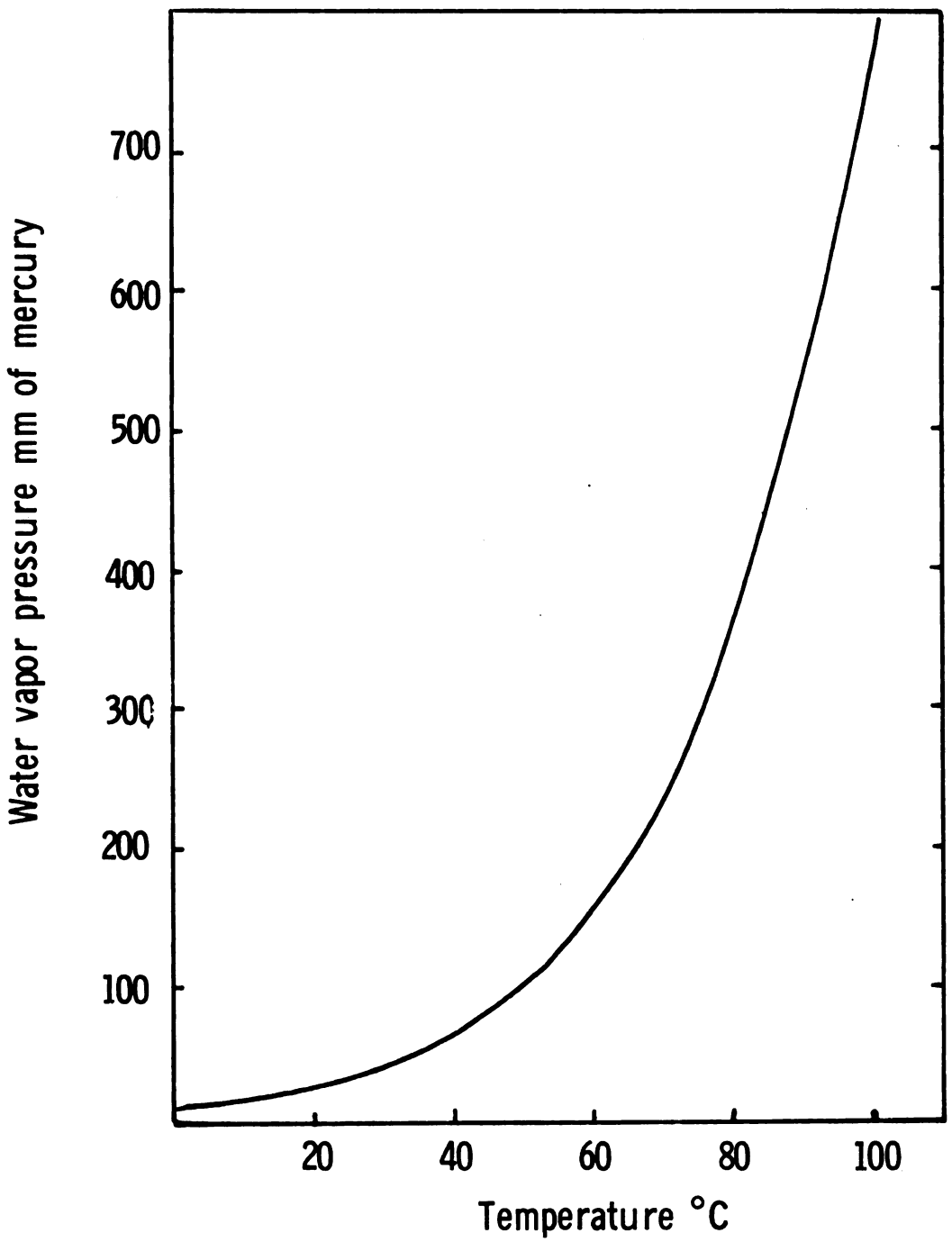

FIGURE 6. Vapor pressure changes of water between 0 and 100 C.

Determination of activation energies and entropies

On the basis of D values obtained at 10 and 21 C and at the various RH values, activation energies, ΔE, have been estimated, by making use of the relationships:

$$\frac{\text{Log } D_2 - \text{Log } D_1}{T_1 - T_2} = \frac{1}{Z}$$

and

$$\Delta E = \frac{2.303 \; R \; T_1 \; T_2}{Z}$$

R = Gas constant cal/mole; Z = increase in temperature necessary to reduce D by one log cycle. D_1 and D_2 are decimal reduction times at the corresponding absolute temperatures (T_1 and T_2 resp.).

Entropy changes associated with bacterial death were calculated by using Frossling's equation (64):

$$\Delta S = \frac{\ln K - \ln\left(\frac{K_b}{h}\right) - \left(\frac{H}{RT}\right)}{R}$$

S = activation entropy
K = death rate
K_b = Boltzmann's constant
h = Planck's constant
H = Heat of activation assumed equal to E
R = Gas constant cal/mole
T = Absolute temperature

The Arrhenius plots and corresponding activation energies and entropies are shown on Figure 7, and Table 2, respectively. The activation energies for S. newbrunswick resemble those obtained for S. marcescens by Webb (151) in that they increase from initial to secondary period of death at the respective RH levels, probably signifying that bacteria surviving longer may require more energy for their destruction.

The negative value of the activation entropy (Table 2) seems to indicate concentration of solutes as a result of water evaporation is probable during the airborne state. The entropy values decreased from initial to secondary periods of death, as shown by their larger negative values (Table 2). This may further indicate a concentration of solutes or tightening of molecules as a result of increasing evaporation of water from the bacteria during aerosol storage.

The decrease in entropy during the airborne state resulting from either concentration of internal solutes or tightening of macromolecules or both may signify considerable alteration in the physical arrangement of intracellular entities. Alteration of tertiary structures

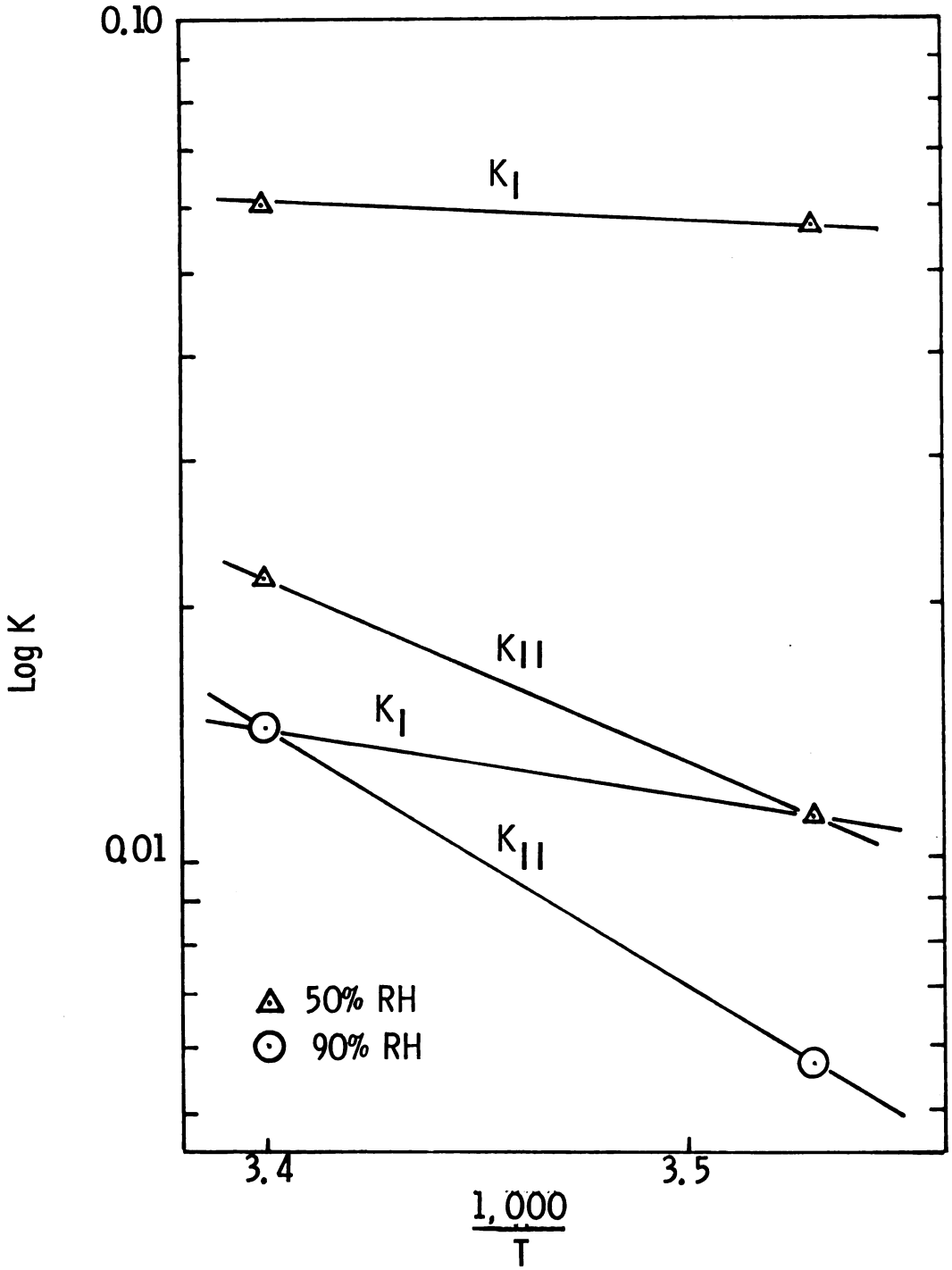

Figure 7. Arrhenius plots of initial (K_I) and secondary (K_{II}) death rates of airborne Salmonella newbrunswick at 50 and 90% RH. (T) absolute temperature, K.

Table 2.--Activation energies and entropy changes associated with death of airborne Salmonella newbrunswick.

% RH	Calories/mole		Entropy units	
	E_I	E_{II}	S_I	S_{II}
30	7357	8649	-21	-24
50	8760	9670	-23	-25
70	6230	10775	-21	-26
90	3520	13360	-17	-27

E_I, E_{II} are activation energies during initial and secondary periods of death;

S_I and S_{II} are entropy values for the same periods, resp.

of enzymes, ribosomes, genetic material (DNA) as well as the cytoplasmic membrane during the airborne state and subsequent rehydration may profoundly affect the normal functions leading to loss of viability of bacteria.

Gradual rehydration of airborne *S. newbrunswick*

The rate of increase in weight of *S. newbrunswick* during gradual rehydration tended to follow a regular pattern as shown in Figures 8 and 9. The rate of increase was relatively slow at the lower RH at about 60-70%. At higher RH the weight began to increase sharply. The control filter without bacteria remained constant in weight.

Webb's experiments (144) have shown a considerable loss of water from *S. marcescens* cells at higher RH levels where death rate was very small. However, increased death rates were associated with lower rates of water loss at lower RH levels. The lower rates of water loss (144) were claimed to arise from loss of tightly held bound water of vital macromolecules. Conversely, the rehydration pattern is somewhat similar. It is marked by a slow weight gain at lower RH levels and an increase at higher RH levels. An increased uptake of water at higher RH levels could lead to rupture of the cell membrane and subsequent leakage of cellular substances.

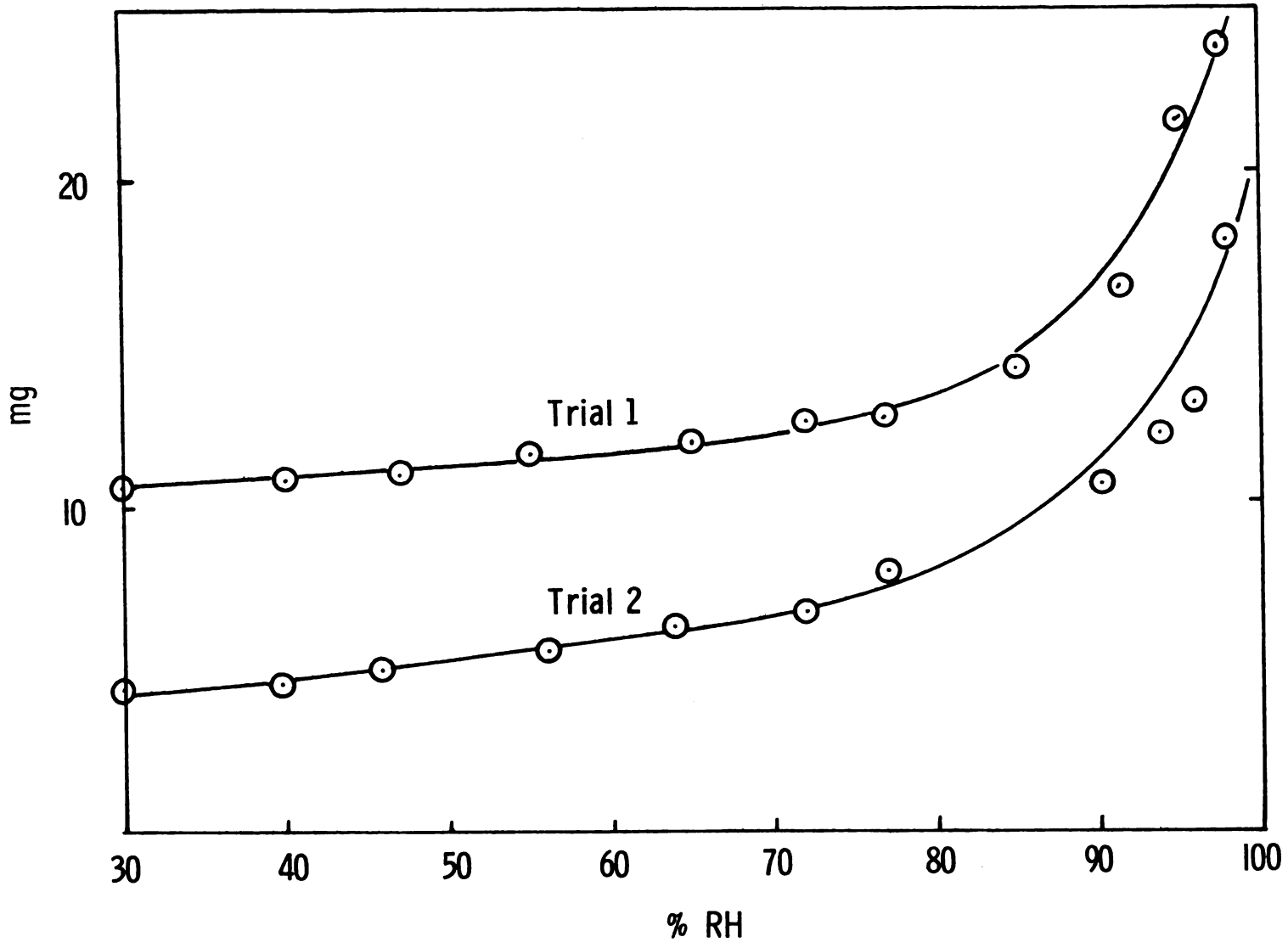

FIGURE 8. Changes of weight of airborne Salmonella newbrunswick during rehydration from 30% RH at 26.4 C.

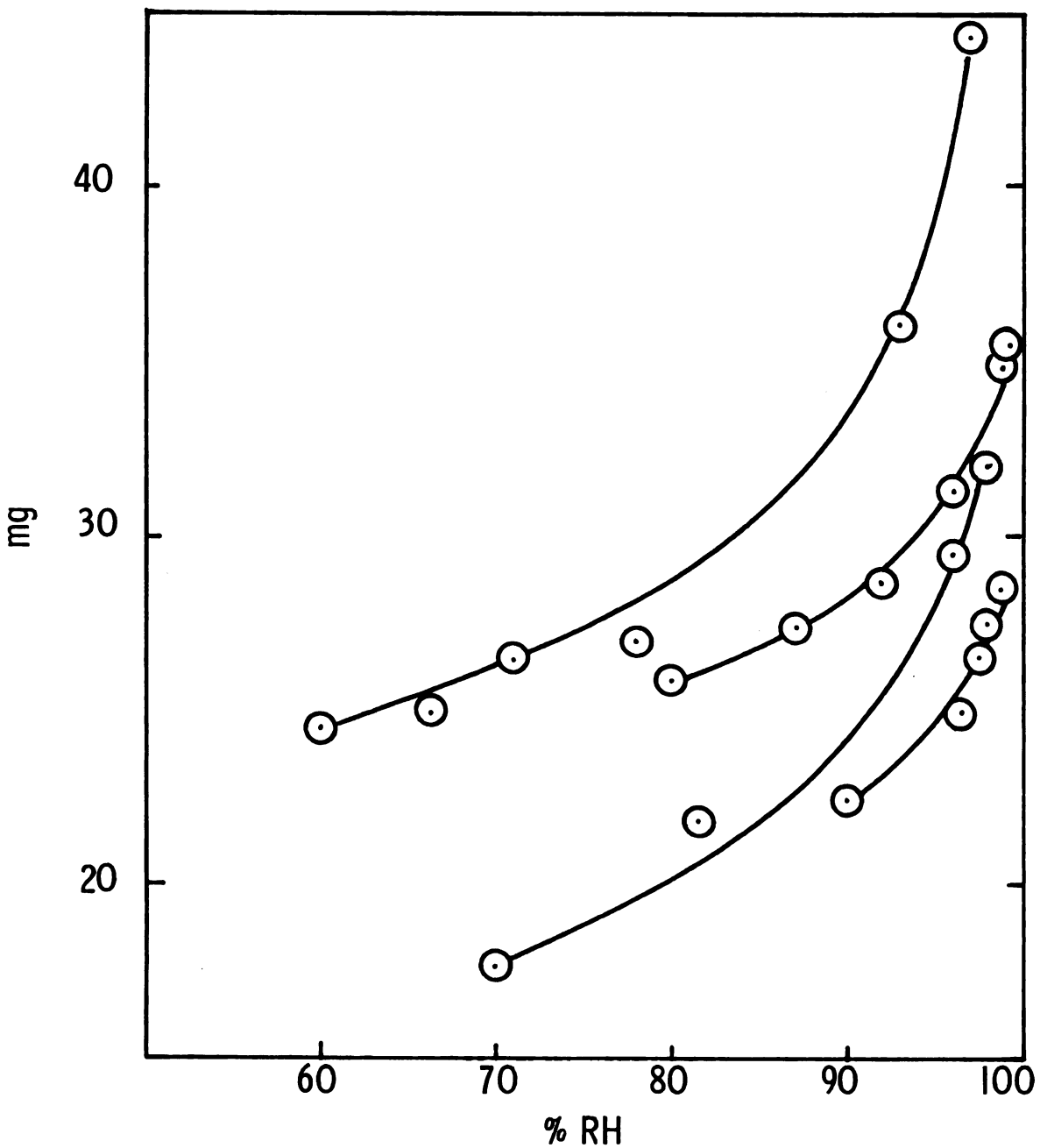

Figure 9. Changes of weight of airborne Salmonella newbrunswick during rehydration from 60, 70, 80 and 90% RH at 26.4 C.

Leakage materials from airborne S. newbrunswick

The release of various leakage materials from airborne S. newbrunswick as a result of gradual and rapid rehydration was investigated in order to observe if there was permeability damage as a result of aerosolization. Analyses of types of materials released were used to infer the types of biochemical changes that might take place and affect microbial viability in the airborne state.

Supernatant fluids of the rapidly and gradually rehydrated samples of S. newbrunswick collected on filters were analyzed for the presence of various materials. The results in Table 3 indicate that the leakage materials tested were present in varying amounts depending upon RH levels. Since these tests (56, 57) were qualitative, arbitrary units (-,?,+,++) were assigned to the reactions depending on their strength. Generally, less leakage of materials appeared at 30 and 90% RH than at other relative humidities tested.

Leakage of inorganic ions

Potassium (Table 3) was present in at least one of the two trials at most of the RH levels from 30 to 80%.

Table 3.--Leakage of materials from airborne Salmonella newbrunswick rehydrated with various relative humidities.

Leakage Materials	Trial	% relative humidity														
		30			40			50			60			70			80			90		
		C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R	C	S	R
Inorganic Ions																						
Potassium	A	?	+	+	?	+	+	?	?	?	-	-	-	?	-	?	?	+	+	-	-	-
	B	?	+	+	?	+	+	?	?	?	-	-	-	?	?	?	-	-	-	-	-	-
Sodium	A	?	?	?	?	?	?	?	?	?	?	?	?	?	-	-	?	?	?	-	-	-
	B	?	?	?	?	?	?	?	?	+	?	?	?	?	-	-	?	?	+	-	-	-
Magnesium	A	-	?	+	?	+	++	?	+	+	?	+	+	-	+	+	?	+	++	-	-	-
	B	?	+	++	?	+	+	?	+	+	?	+	+	-	+	+	?	+	+	?	+	+

Table 3.--Cont.

Phosphate	A	-	-	-	-	+	++	-	-	-	-	-	-	?	+	+	?	+	++	-	-	-
	B	-	-	-	-	-	+	-	+	+	-	-	-	?	?	?	-	-	+	-	-	-
Organic Materials																						
Simple Carbohydrates	A	-	-	-	?	+	++	-	-	-	?	++	++	-	?	?	-	+	+	-	?	?
	B	-	-	-	?	+	++	-	?	?	+	+	+	-	?	?	-	+	+	-	?	?
Amino acids	A	-	?	?	-	-	-	?	+	++	?	+	+	-	+	+	?	+	+	-	-	-
	B	-	-	-	?	+	++	-	+	+	?	+	+	-	+	++	?	+	+	-	+	+
Protein-like substances	A	-	-	-	-	?	-	-	-	-	-	-	-	-	?	?	-	?	?	-	-	-
	B	-	-	-	-	?	-	-	?	?	-	-	-	-	?	?	-	-	-	-	-	-

C = control; S = slowly rehydrated; R = rapidly rehydrated.

However, at 60 and 90% potassium may have been present below the sensitivity of the test. The presence of sodium was marked by weak reactions at all of the RH levels indicated except at 70 and 90% relative humidity where there was no reaction to the test for sodium. Tests for phosphates were positive at 40, 50, 70, and 80% RH to varying degrees in both rapidly and slowly rehydrated samples. Magnesium was present in both the rapidly and slowly rehydrated samples at all RH values in varying amounts.

The internal ionic environment of a bacterium is of basic importance in maintaining its normal metabolic functions. Potassium is required (102) to activate a labile SH-enzyme for the formation of peptide bonds during protein synthesis. Bowen et al. (18) reported that aggregation of ribonucleoprotein in washed E. coli depends on concentrations of phosphate and magnesium ions present. Horiuchi et al. (87) observed the degradation of ribosomes in a phosphorus-deficient environment. Although for short periods the smaller components formed by disaggregation remain in the cells, leakage of RNA into buffer solutions has been reported by Rotman (124). Wade (137), investigating the role of magnesium as a stabilizer of RNA in

E. coli, identified two routes of breakdown. The M route requires magnesium and leads to nucleoside 5'-phosphates, while the magnesium-independent V route yields, eventually, nucleoside 3'-phosphates. Davis and Feingold (39) reported that the beginning of the membrane damage of streptomycin-treated E. coli was marked by potassium leakage.

Attardi (8) reported that potassium and sodium along with magnesium play a role in the neutralization of the excess negative charges of phosphate groups of ribosomes. The importance of potassium and sodium in membrane transport systems have been reviewed by Mitchell (111) and Kepes and Cohen (89). However, Anderson and Dark (5) observed that airborne E. coli, after rehydration, may lose most of its potassium without losing its viability. Therefore, they concluded, loss of control of potassium in the metabolism is not immediately lethal to the organism provided it is returned to the medium containing adequate supply of potassium.

The severely decreased ability to synthesize inducible protein in airborne E. coli after recovery observed by Anderson (2) correlated well with the loss of

potassium after aerosol recovery. The evidence obtained by Ennis and Lubin (55) indicates that potassium ions are responsible for normal ribosomal function in a mutant of E. coli. A specific monovalent ions to magnesium ratio has been reported to be necessary for the correct functioning of ribosomes in E. coli (23). Sodium, potassium, or ammonium ions are required for various enzymes for the stabilization of the particular conformation responsible for their maximal catalytic activity (102).

Anderson et al. (6) found that when airborne Aerobacter aerogenes was stored for 1.2 sec it released most of its labeled potassium (^{43}K) at 40% RH. The release of potassium decreased as the RH of the atmosphere increased. During a 5-min aerosol storage however there was an intermediate range of RH between 80 and 60% where the release of potassium was lower than at other RH values. With various E. coli strains they observed the lowest potassium release at 1.2 sec aerosol age, 100 to 90% RH and nearly 100% release of potassium at the lower RH levels. The release of potassium at all RH levels tended to be near 100% of the original ^{43}K content. However, Staphylococcus epidermidis NCTC 7291 appeared to

lose less potassium as a result of the 1.2 sec, 5 and 30 min aerosol storage periods between 100 and 80% RH than at lower % RH values. These observations seem to indicate that differences among bacterial species do exist in their potassium release during rehydration from the airborne state at different RH values.

The appearance of leakage materials in the supernatant fluids of rehydrated airborne S. newbrunswick undoubtedly indicates changes in the permeability of cells. The low leakage of material at 90% RH is also correlated with lower death rate of S. newbrunswick at this level of RH (Figures 5, 7). However, at other RH levels no direct correlation could be established between death rates and the appearance of leakage materials.

Leakage of organic materials

Table 3 shows the appearance of organic materials in the supernatant fluids of aerosolized S. newbrunswick rehydrated from various relative humidities. Proteins gave only very weak reactions at 40, 50, 70, and 80% RH. The significance of this observation is probably that the

plasma membrane is not sufficiently damaged to permit the release of large molecules.

Simple carbohydrates, detected by periodate oxidation without heating, appeared to be present at 40, 60, and 80% RH in both treatments. At other RH values there was little or no reaction indicating simple carbohydrates. These substances may be derived from internal pools of carbohydrate metabolic products, or may be associated with the nucleosides from their breakdown or the precursors of necleotides such as ribose or desoxyribose. There appeared to be little or no amino acid leakage at 30, 40, and 90% RH whereas weak to moderate reactions were observed at intermediate RH levels of 60 to 80%. The loss of some amino acids during rehydration may seriously affect recovery of the organism after aerosolization.

During aerosolization of S. newbrunswick, both amino acids (Table 3) and especially UV light absorbing materials were detected in the supernatant fluids of rehydrated cells. Cleaves and Cohen (24) have shown that during magnesium starvation of E. coli B, for example, most of the ribosomes break down to low molecular weight components and, when magnesium is restored to the medium, the

cells recover. However, the rate of recovery is increased by a supply of 21 amino acids. Nevertheless, only two of the amino acids, histidine and methionine, stimulated the accumulation of the cellular RNA, and the rest of the amino acids increased protein synthesis. Consequently, even minor losses of essential amino acids during rehydration after aerosolization may result in the inability of bacteria to recover. Wagman (138) presented evidence of cytoplasmic membrane injury as a result of freeze drying of bacteria. Rehydrated suspensions of E. coli from the freeze-dried state showed a decrease in viable count accompanied by release of ultraviolet light absorbing material and amino acids.

Leakage materials absorbing ultraviolet light at 265 nm

The release of 265 nm absorbing material from airborne S. newbrunswick showed about a tenfold increase over the unaerosolized control at various RH levels (Figure 10). The significance of the 265 nm absorbing material is that it may conceivably be associated with nucleic acids or may result from their breakdown. Supernatant fluids of both

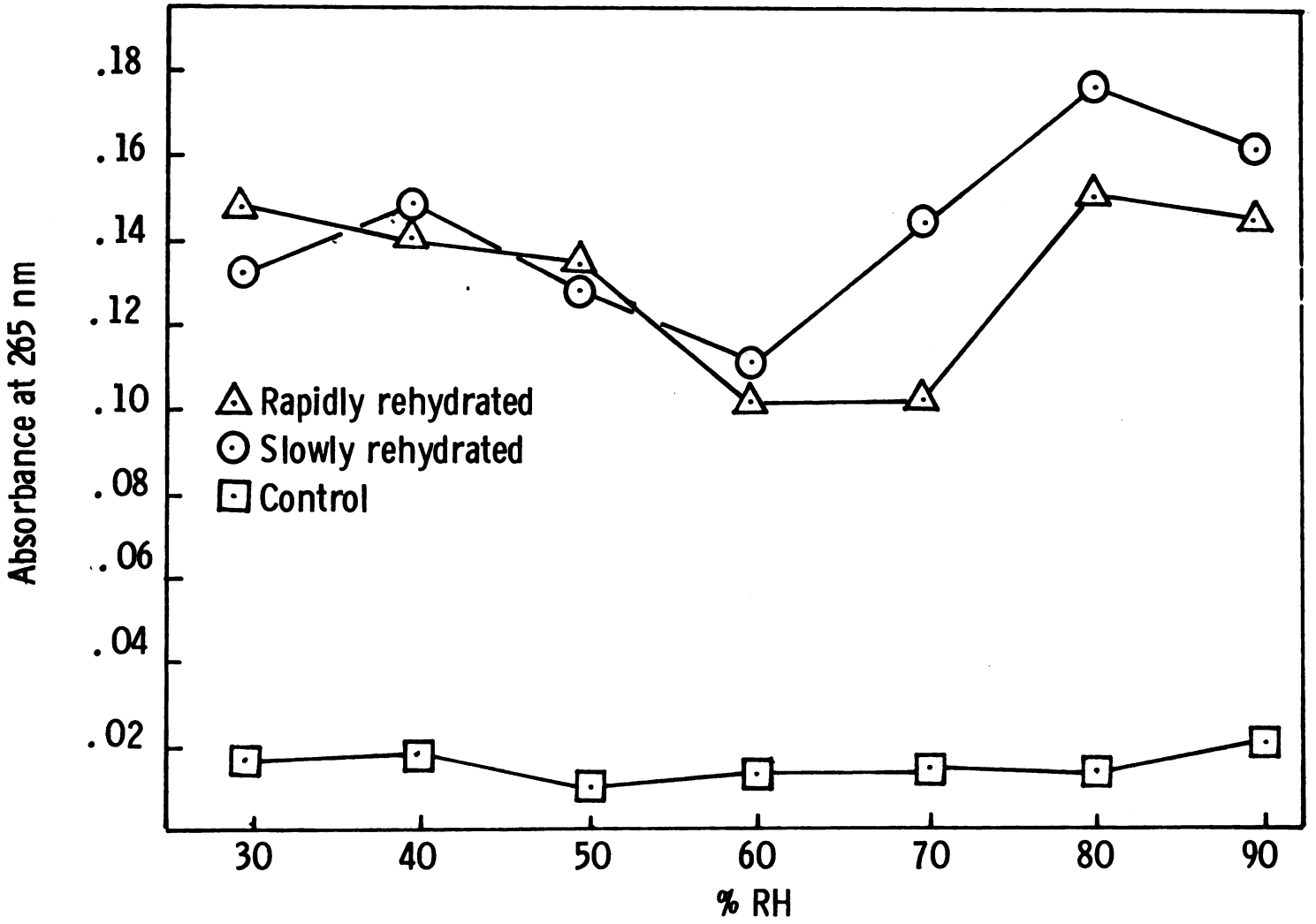

Figure 10. Release of 265 nm absorbing materials from airborne Salmonella newbrunswick rehydrated at various RH levels and 26.4 C.

slowly and rapidly rehydrated samples (Figure 10) contained significant amounts of 265 nm absorbing material when compared to the unaerosolized control. The release of this material was generally higher when the cells were rehydrated slowly than when rapid rehydration took place. The leakage of the 265 nm absorbing material from rapidly rehydrated cells was slightly greater at 80 and 90% than at 30 to 50% RH and at 60 and 70% there was less release of this material. For the gradually rehydrated sample there was a single low point at 60% RH but the leakage curves for both slowly and rapidly rehydrated cells were approximately the same.

Cox (32) claimed that more of the RNA was released from E. coli K12 cells when they were aerosolized at 80% RH than at 40% RH. He had no explanation for the phenomenon. Slowly rehydrated samples resulted in just as high or higher quantity of leakage material absorbing at 265 nm than samples rehydrated rapidly or by liquid impingement. The author hypothesizes that the damage causing these changes may be due to the dehydration during the airborne state and not necessarily to the means of rehydration. Aerosolization at 80% RH compared to aerosolization at

lower RH resulting in slightly greater release of leakage material may indicate that sufficient water is present in the bacteria at this RH level to permit enzyme action while airborne. Aerosolization at 40% RH might result in the removal of a greater amount of water from the bacteria and a dryer state would cause decreased enzyme action. Therefore, ribosomes in the bacteria that were rehydrated from 40% RH may be in a lesser state of decomposition than those recovered from 80% RH. This reasoning is proved to some extent by Cox's data (32) showing that upon recovery at approximately 0 time the airborne bacteria rehydrated from 80% RH show about twice as much ^{14}C related to RNA breakdown than that rehydrated from 40% RH.

Figure 11 a-g shows an attempt of chromatographic separation of the 265 nm absorbing material from the supernatant fluid of S. newbrunswick rehydrated from various relative humidity levels. Reference compounds shown in 11 h indicate that probably the molecular size of the majority of the molecules composing the 265 nm absorbing material was comparable to that of adenosine monophosphate, a mononucleotide. Thus, one may hypothesize

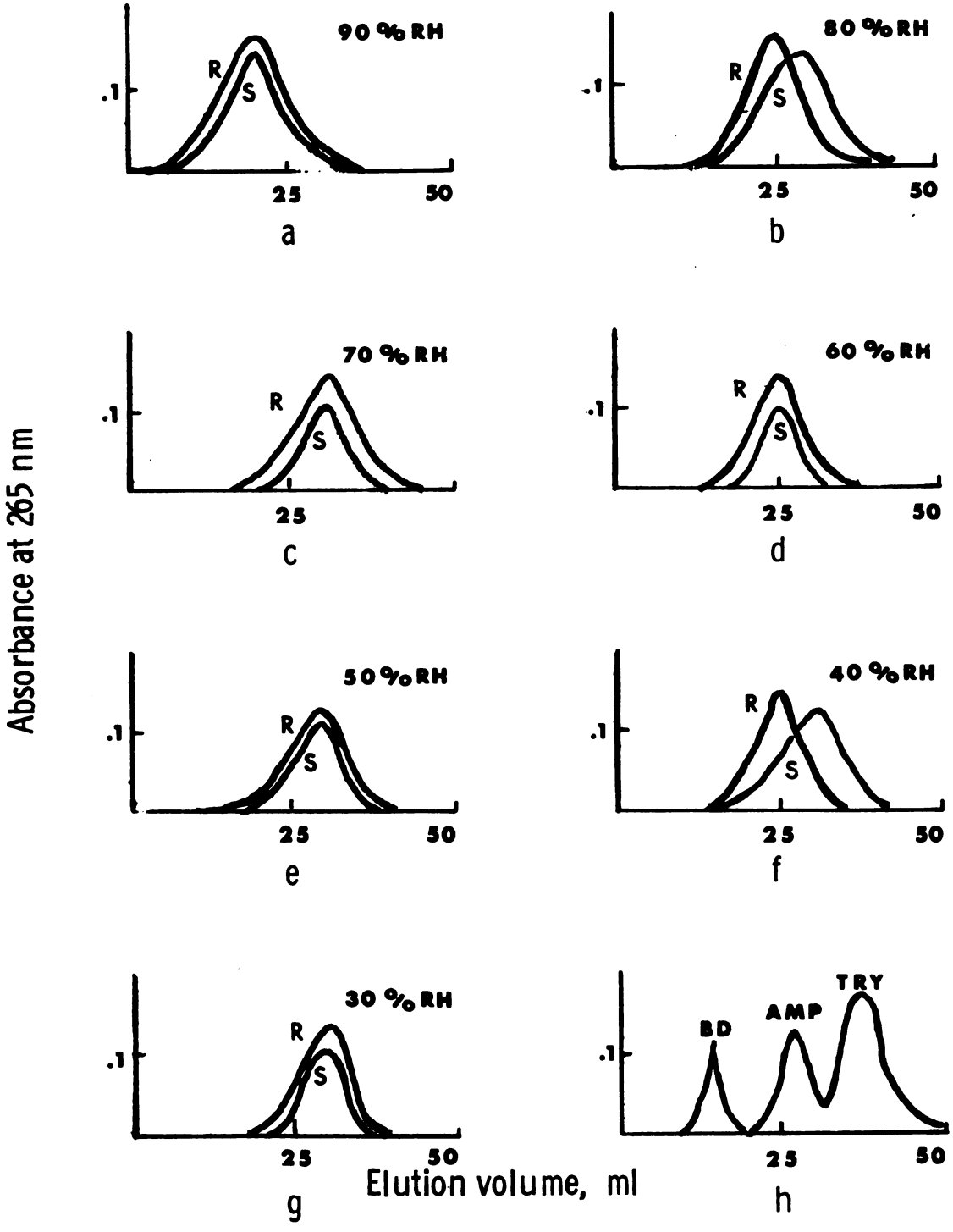

Figure 11. Chromatographic separation of 265 nm absorbing leakage material from airborne Salmonella newbrunswick. (S), (R): Slow and rapid rehydration, resp. (BD) blue dextran, (AMP) adenosine mono phosphate, (TRY) tryptophane.

that either the membrane is not sufficiently damaged to release larger compounds or that the hydrolysis of macromolecules containing 265 nm absorbing material is complete enough to produce molecules of the size of mononucleotides.

The release of magnesium during rehydration from the airborne S. newbrunswick is probably the most serious loss since it may bring about the degradation of ribosomes. This is shown by the increase of 265 nm absorbing material (Figure 10) in the supernate. Mandelstam (105) found no breakdown of E. coli ribosomes suspended in the presence of 0.01 M magnesium. However, in the absence of magnesium there was a linear breakdown of RNA which was indicated by the appearance of material absorbing at 260 nm. Maltman (103) showed a broad band (extending from 230 to 290 nm) of absorption for the leakage material in the UV spectrum (with a peak at 260 nm) for dried and rehydrated S. aureus. Bolton et al. (16) reported that ribonuclease of ribosomes was activated when ethylenediaminetetracetic acid chelated magnesium ions or when magnesium ions were displaced on the ribosomal complexes by 0.125 M sodium chloride, all the RNA became acid soluble in about 20 min. It was concluded that in the

absence of magnesium ions the uninhibited ribonuclease destroyed the ribosomes.

Strange and Shon (132) reported that magnesium ions protected thermally injured A. aerogenes. Sodium chloride was deleterious probably because sodium ions displaced magnesium ions. Addition of magnesium ions (0.5 M) to diluents in which bacteria were heated markedly decreased death rate. The same authors showed that at least 80% of the total UV light absorbing leakage components of the thermally injured cells diffused through cellophane during dialysis against phosphate saline buffer. This indicated that the components were of a low molecular weight. They have shown that potassium and inorganic phosphate also leaked from bacteria during heating. By their experiments they demonstrated that initial loss of RNA occurred without being accompanied by loss in viability. This loss of viability during heating was more closely related to time. A loss of 38% RNA in starved bacteria resulted in less leakage of RNA and greater viability on heating at 47 C than in non-starved bacteria. Therefore, the amount of 265 nm absorbing leakage materials or perhaps of other leakage

substances are not necessarily proportional to loss of viability. Sogin and Ordal (130) have noticed ribosomal breakdown, during heating, which resulted in considerable size reduction of RNA as shown by chromatography on methylated albumin kieselguhr column.

Gritsavage's investigations (73) showed that freezing and thawing of E. coli (ATCC #11303) caused release of pentose, an ultraviolet light absorbing product, and protein-like substances. The UV light absorbing material was shown to be a monophosphate. He also demonstrated that the amount of mononucleotides was not increased by adding ribonuclease to the supernates confirming that there were no polynucleotides present. Membrane damage was marked by the leakage of materials similar in nature to those of aerosolized S. newbrunswick. Roszman (123) demonstrated that cellular injury of E. coli (ATCC #11303) resulted during freezing. The injury was marked by leakage of materials having a maximum absorption at 260 nm in the UV spectrum. The leakage materials had a restorative effect on frozen cells during recovery. A loss in viability was related to leakage of the 260 nm absorbing material. The cell injury was related to the

release of UV light absorbing material. The vital importance of the leakage material was shown by its restorative effect on cells when injured cells are added to the leakage material. Thus the leakage materials may be closely related to life or death of injured bacteria.

These findings corroborate experimental results presented here. RNA loss is associated with an increase in UV light absorbing material that appears in the suspending medium of injured cells. The degree of RNA loss is not necessarily related to loss of viability of bacteria. The findings of Strange and Shon (132) also indicate that the reduction in size of RNA in thermally injured bacteria is considerable which was shown by at least 80% diffusibility of UV light absorbing material through cellophane membranes.

Kozloff (93) showed that a deoxyribonuclease inhibitor is RNA in nature; therefore, it is possible that the stability of DNA depends upon the stability of certain RNA. Mandelstam (105) also suggests that ribosomal breakdown might release proteolytic enzymes which might be absorbed on the ribosomes in the intact cells. Consequently, release of magnesium from rehydrated airborne cells may lead to fragmentation of ribosomal RNA by

ribonuclease, may remove inhibitor of deoxyribonuclease, and release some proteolytic enzymes. The end results of these may be reflected by the appearance of small molecular weight 265 nm absorbing material as shown by chromatographic separation of supernates of rehydrated airborne S. newbrunswick, and by the presence of amino acids and protein-like substances as shown in Table 3.

Electron microscopic results

The unaerosolized cells of S. newbrunswick which were suspended in distilled water prior to fixation and embedding seem to have undergone plasmolysis during preparation as shown by Figure 12. The aerosolized and rapidly or slowly rehydrated cells which have been freeze-dried subsequently show some clear areas (Figure 13 A and B, mark X) which may represent depletion of ribosomes. In the cells which were aerosolized and freeze-dried before fixation (Figure 14) the ribosomes appear as dark granules (longitudinal section) nearly filling the entire cell. The clearing of areas marked by X on Figure 13 A and B and the leakage of UV light absorbing materials (Figure 10) appear to support the hypothesis that partial destruction

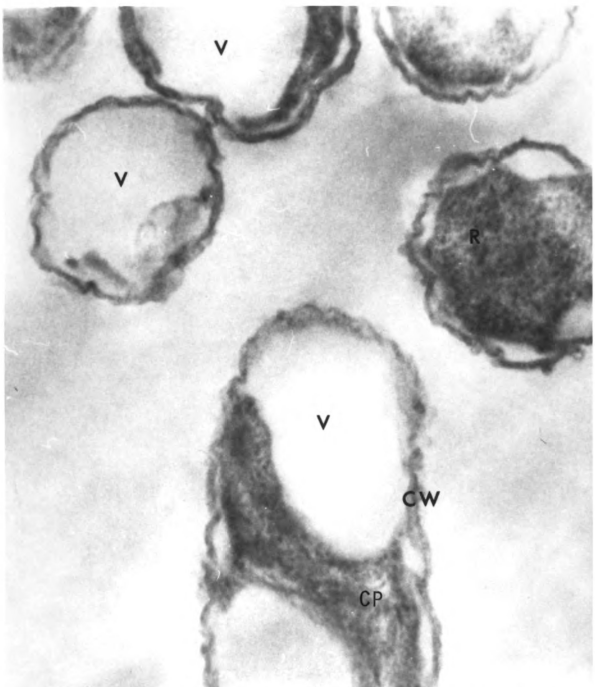

Figure 12. Electron micrograph (95,000 X magnification) of the unaerosolized Salmonella newbrunswick. (V) vacuolation; (CP) cytoplasm; (CW) cell wall.

Figure 13. Electron micrograph (100,000 X magnification) of aerosolized and rapidly (A) or slowly (B) rehydrated and subsequently freeze dried cells of Salmonella newbrunswick. (CW) cell wall, (R) ribosomes, (X) areas of diminished ribosome content.

CW
X
X
X
X
X
X
CW
X
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X
X
B
A

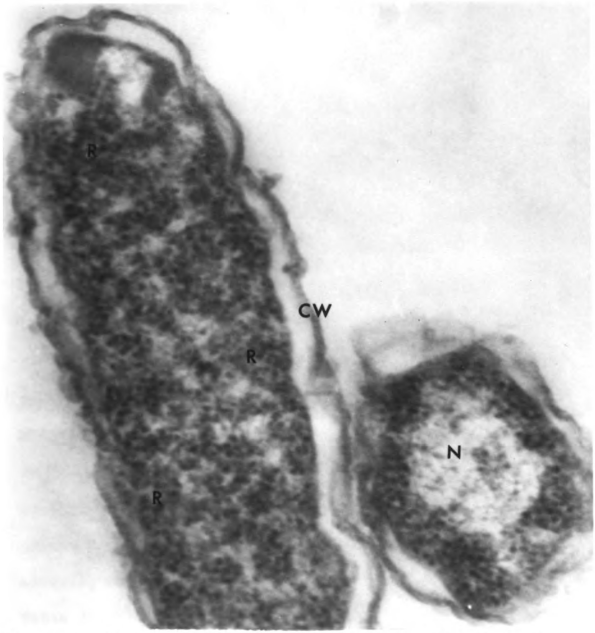

Figure 14. Electron micrograph (100,000 X magnification) of aerosolized, freeze dried, non-rehydrated cells of Salmonella newbrunswick. Longitudinal and cross sections. (R) ribosomes, (CW) cell wall, (N) nuclear filament.

of ribosomes takes place as the result of aerosolization, rehydration, and freeze drying. However, when the cells were aerosolized, freeze-dried but not rehydrated, the partial destruction of ribosomes was not observed.

The influence of a bipolar-oriented electrical field on airborne Salmonella newbrunswick and other common species

In two sets of experiments (Table 4) the effect of bipolar-oriented electrical field was determined on distilled water aerosolized S. newbrunswick.

Comparison of death rates (K_D) in Table 4 indicates that usually there was an increase for the chamber in which the electrode was operated (K_D = 0.083) vs. the control experiments where the death rate was lower (K_D = 0.061). The increase in death rate, taking the control as 100%, was 36% during the period. The slopes of the decay curves for total counts determined by radioactivity were also expressed as K_P values given in Table 4. The rate of physical loss K_P increased by 78%. Accordingly, the physical effect of the field appeared to

Table 4.--Death rates of airborne Salmonella newbrunswick under the influence of the bipolar-oriented electrical field.

Test No.	Test Chamber 10,000 volt field		Control Chamber no voltage		Difference	
	K_D	K_P	K_D	K_P	K_D	K_P
1	0.046	0.0767	0.041	0.0576	0.005	0.0191
2	0.085	0.135	0.053	0.0640	0.032	0.0710
3	0.076	0.115	0.069	0.0658	0.007	0.0492
4	0.096	0.121	0.056	0.0658	0.040	0.0552
5	0.115	0.135	0.089	0.0742	0.026	0.0608
Average	0.083	0.1165	0.061	0.0655	0.022	0.0510

K_D death rates.

K_P physical decay rates.

be about twice as great on the airborne S. newbrunswick as the effect on the viability.

Similar experiments for S. marcescens did not yield the significant change (Table 5) in the death rate under the influence of the electrostatic field. K_D values were 0.330 ± 0.1664 and 0.3979 ± 0.2052 for test and control chambers, resp. The physical decay in the test

Table 5.--Death rates of airborne Serratia marcescens with a bipolar-oriented electrical field.

Test No.	Test chamber 14,000 volts		Control chamber no voltage	
	K_D	K_P	K_D	K_P
163	0.056	1.954	0.088	0.328
164	0.181	1.075	0.268	0.404
165	0.256	0.818	0.311	0.436
166	0.449	1.347	0.697	0.959
167	0.328	1.262	0.802	0.328
168	0.499	1.200	0.529	0.746
169	0.171	1.084	0.161	0.273
170	0.701	1.452	0.572	0.811
171	0.310	1.016	0.603	0.772
172	0.282	0.991	0.436	0.663
173	0.466	1.061	0.397	0.397
174	0.273	1.145	0.249	0.420
175	0.519	0.251	0.211	0.427
176	0.245	0.780	0.339	0.507
177	0.219	0.928	0.305	0.478
Average ± σ:	0.3303±0.166	1.0909±0.368	0.3979±0.205	0.5299±0.207

chamber was twice as high as in the control:

$K_p = 1.0909 \pm 0.368$, and $K_p = 0.5299 \pm 0.2074$, resp.

On the basis of findings in the above experiments, a series of 5 to 8 hr experiments, with continuous aerosolization, were conducted on several species of industrially important organisms.

Results presented in Figure 15 are typical of those obtained in more than 100 trials. P. fragi was aerosolized for 8 hr. The concentration of the aerosol in the chamber with the bipolar-oriented electrical field with 20,000 volts was consistently lower than in the chamber without the field. The gradual decrease in aerosol concentration after 2 hr may have been due to several factors: 1) decrease of viability of the organism in the distilled water suspension used for spray, or 2) condensation of aerosol on the plastic inlet tube to the chamber. However, these changes and minor variations between control and test chambers were statistically corrected.

The mean percentage reduction values for the five aerosolized species at 6,000, 10,000, 14,000, and 20,000 volts were obtained by using the least squares fitting analysis to determine the most probable relationship between test and control chambers.

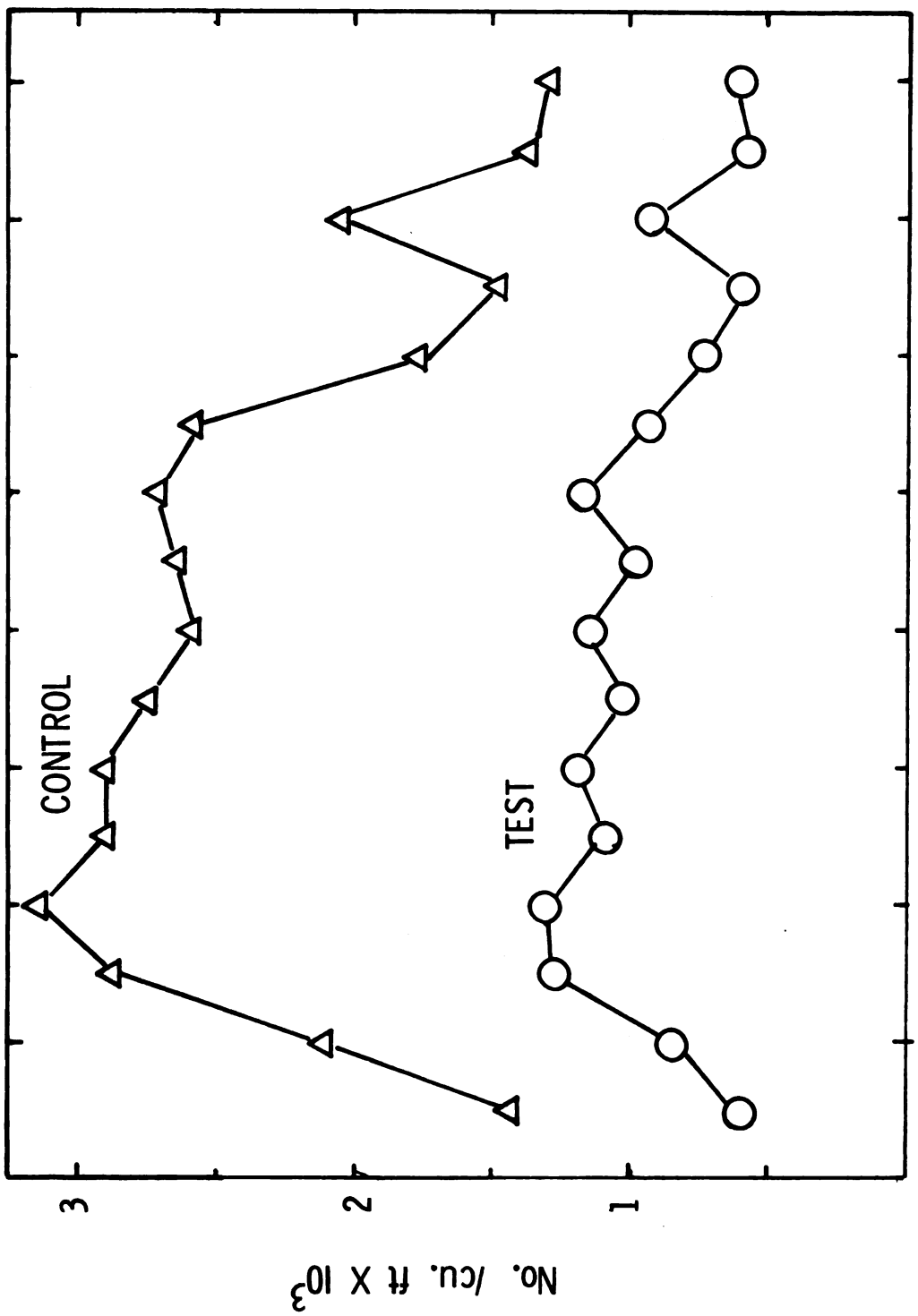

FIGURE 15. Influence of a bipolar electrical field at 20,000 volts on airborne Pseudomonas fragi count.

Figure 16 indicates that an average reduction of 43% of S. marcescens occurred at 14,000 field voltage. At the next higher voltage (20,000) a slight decline in reduction was observed which was within the experimental error. S. marcescens (151) is known to be susceptible to environmental stresses. Therefore, any influence of the bipolar-oriented electrical field on viability would be expected to be emphasized at this voltage compared to lower voltage. As indicated previously, death rate for this organism did not increase under the influence of the bipolar-oriented electrical field. Only the physical decline of the organism was approximately doubled.

The influence of the field voltage was the greatest on P. fragi compared to four other species. The aerosol population of this organism was reduced by a mean of 59% (three to four trials per voltage level) (range 56-63%) at 14,000 volts (Figure 17); trials at 20,000 volts did not increase the reduction.

The reduction of airborne C. lipolytica reached its maximum of 48% at 14,000 volts (range 34-57%) (Figure 18). On the basis of the mean, no additional reduction was observed at 20,000 volts. However, some experiments caused a reduction up to 60%.

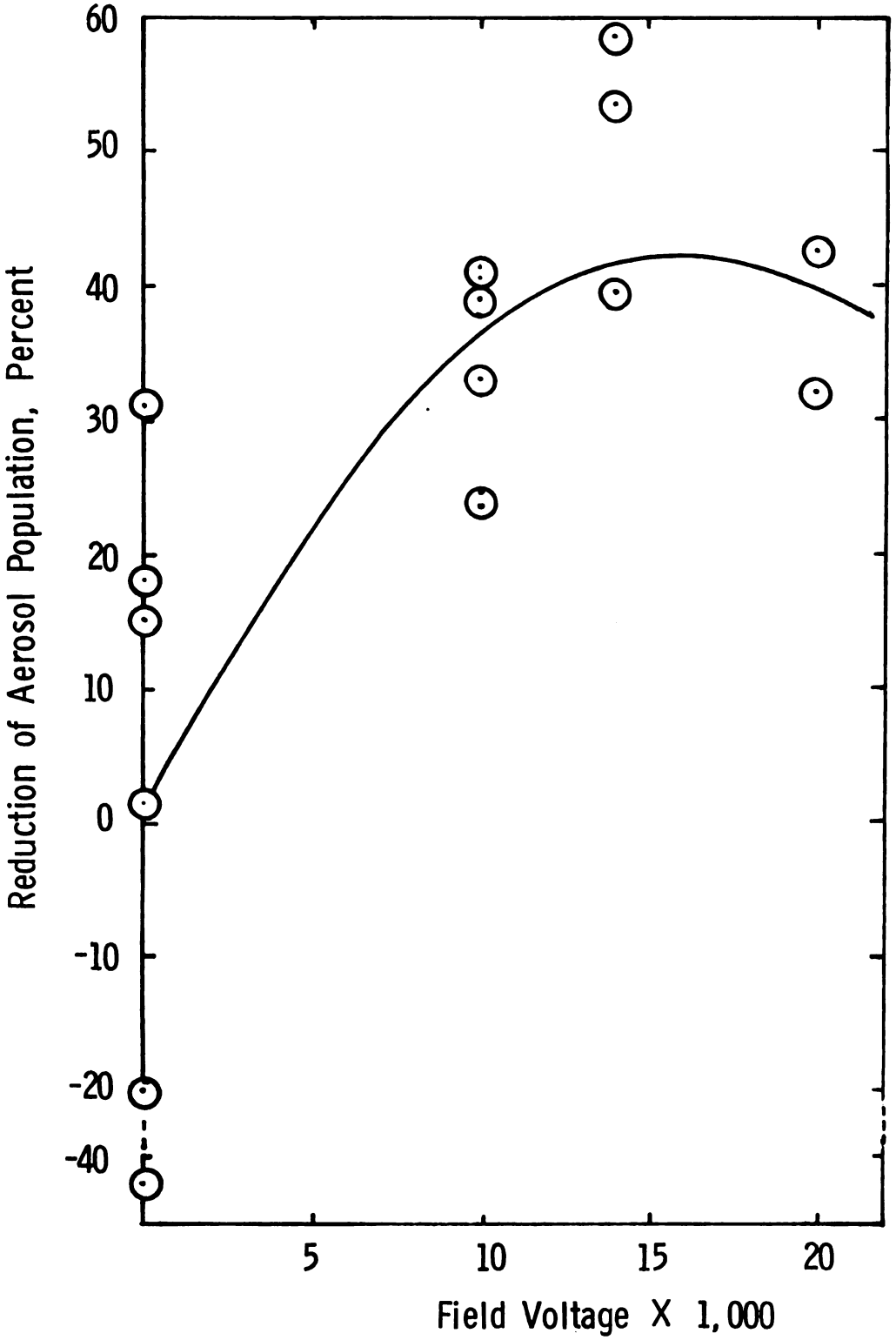

FIGURE 16. Effect of voltage on reduction of airborne Serratia marcescens in a bipolar electrical field.

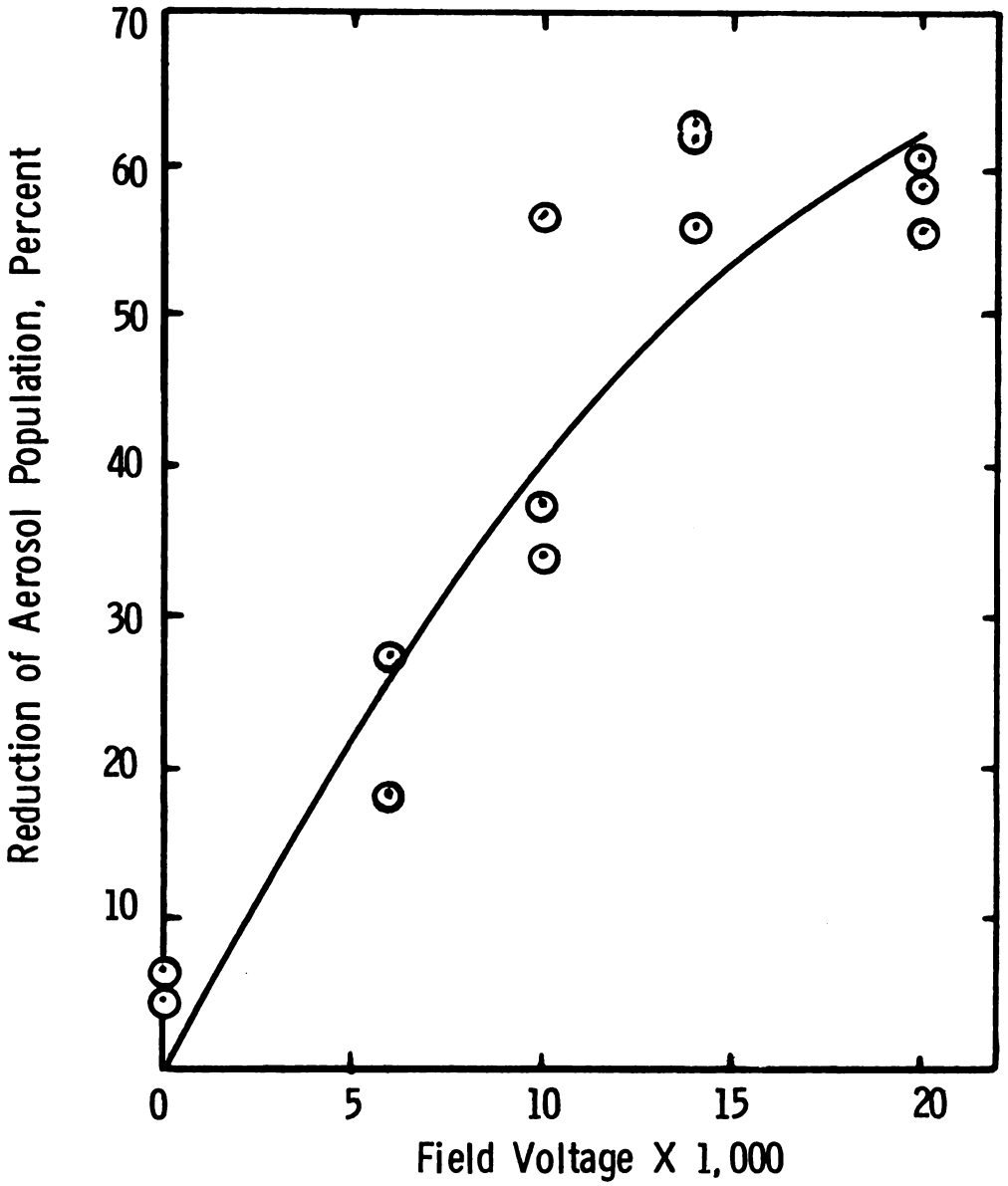

FIGURE 17. Effect of voltage on reduction of airborne Pseudomonas fragi in a bipolar electrical field.

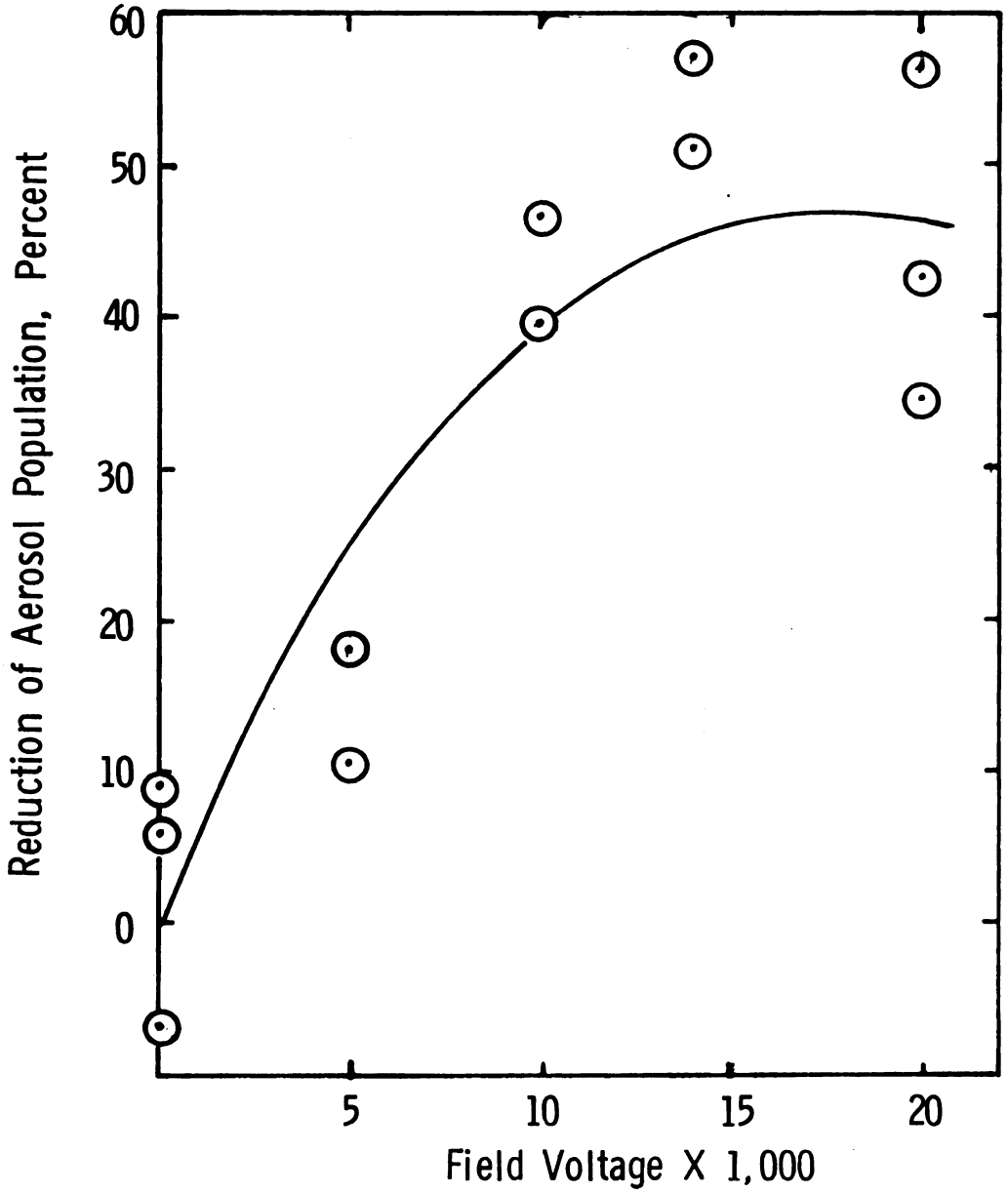

FIGURE 18. Effect of voltage on reduction of airborne Candida lipolytica in a bipolar electrical field.

Airborne P. roqueforti was the least affected (Figure 19) of the organisms studied by the bipolar-oriented electrical field. The average reduction was 37.5% at 20,000 volts (range 31-50%). The fact that the P. roqueforti spores are reduced less by the bipolar-oriented electrical field may be interpreted in two different ways. They might be more inert, by being larger and/or having lesser charges, than other microorganisms under the influence of the bipolar-oriented electrical field or they might be more resistant biologically because they had been in powder form for several months before the trials.

The reduction of airborne B. subtilis spores had a mean (Figure 20) of 52% (range 44-63%) at 20,000 volts. Since the bacterial spores are the most resistant to environmental stresses among the species under study, the results indicated the capacity of the bipolar-oriented electrical field to reduce airborne bacteria would be the most effective if it reduced viability. Results are similar to those of the other microorganisms tested.

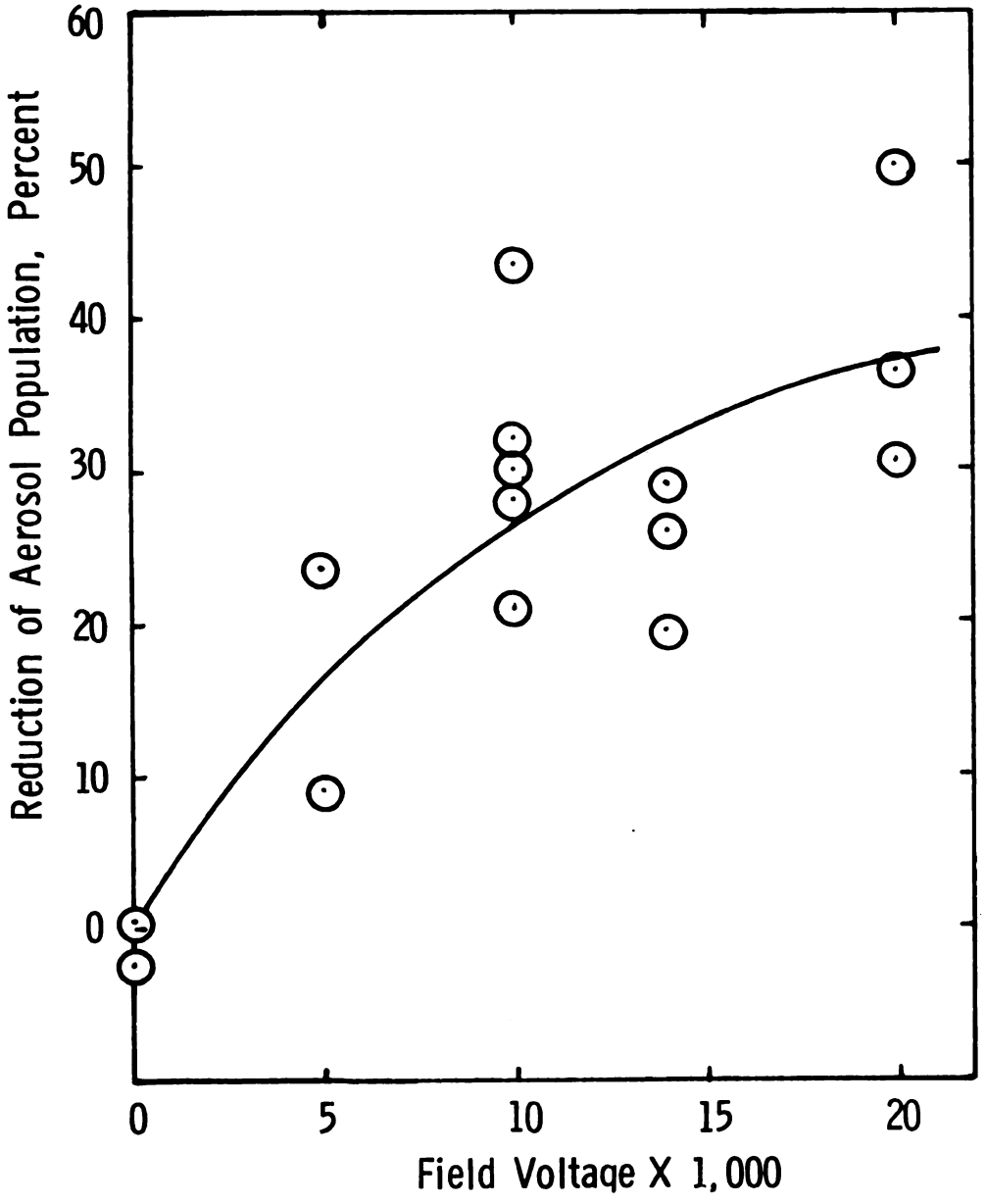

Figure 19. Effect of voltage on reduction of airborne Penicillium roqueforti in a bipolar electrical field.

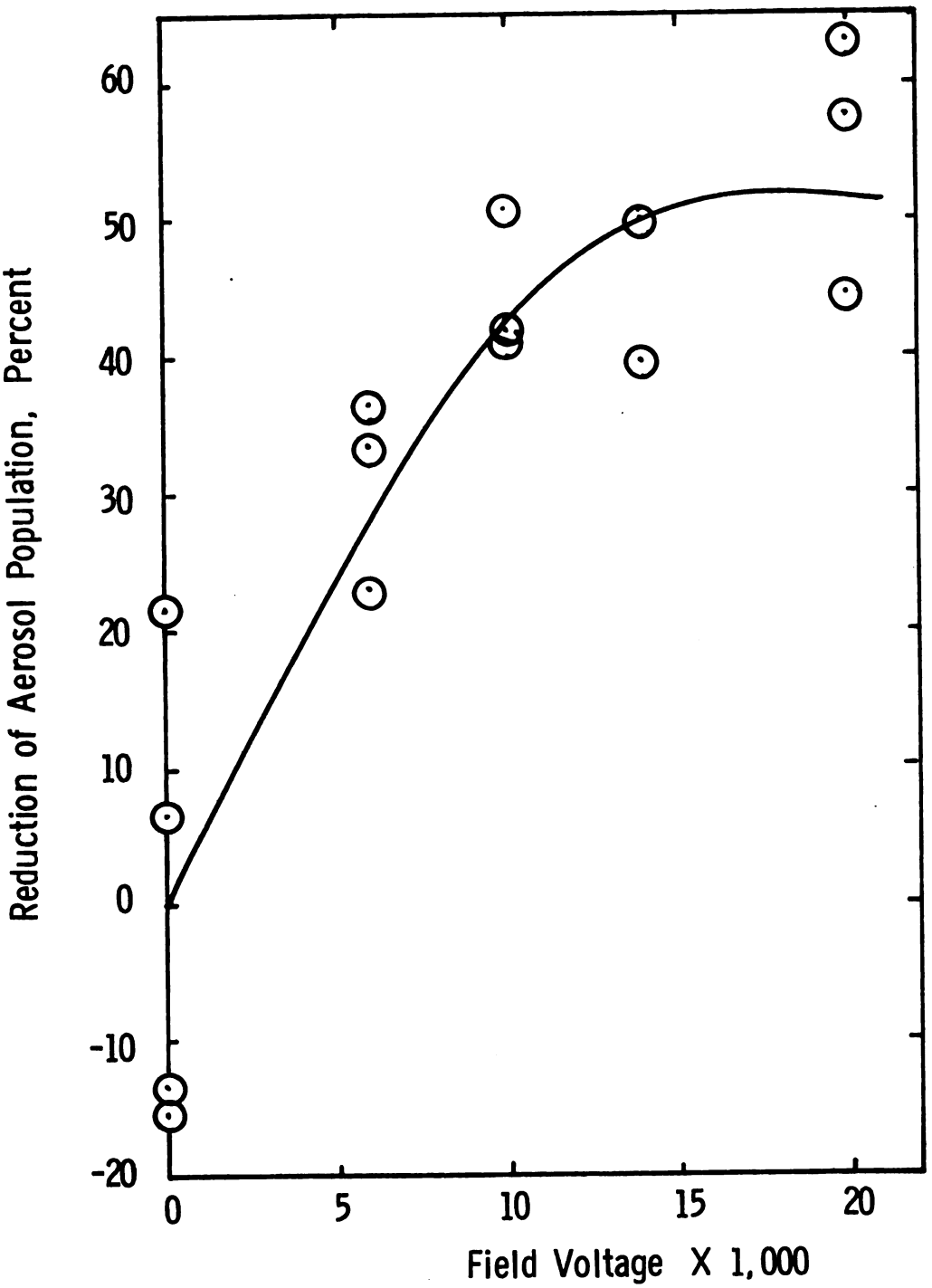

Figure 20. Effect of voltage on reduction of airborne Bacillus subtilis spores in a bipolar electrical field.

Distribution of the deposition of microorganisms from air on the inside surfaces of chambers

The deposition data of airborne S. marcescens, as measured by Rodac plates, on the electrode and door during aerosolization is summarized in Table 6. These results indicate that the electrode with 14,000 volts at 1 hr after aerosolization had three out of five trials with more viable bacteria than the control electrode with 0 voltage. The door of the test chamber had greater deposition, at the same time in five trials, than door in control chamber. The counts were higher at the beginning compared to those at the end of 4 hr on both the charged and uncharged electrodes. However, an increase of deposition was noted on the walls of the chamber with the charged electrode during the 4 hr.

Data of these experiments and others in Table 7 have been evaluated for relative rates of deposition by dividing the surface concentration (number deposited per hr/ft^2) by the corresponding aerosol concentration (no/ft^3). In aerosol sedimentation studies this parameter is referred to as the effective sedimentation rate and is symbolized as ft/hr. It is indicative of velocity.

Table 6.--Rodac plate count on surface of electrode and metal door during aerosolization of Serratia marcescens.

Trial No.	Test chamber 14,000 Volts							Control chamber						
	Initial count/cu ft of air	1 hr		4 hr				Initial count/cu ft of air	1 hr		4 hr			
		Electrode count	Door count	Electrode count	Electrode %	Door count	Door %		Electrode count	Door count	Electrode count	Electrode %	Door count	Door %
88	42,000	436*	614*	7	1.60	246	40.06	40,000	18*	31*	8	44.44	2	6.45
89	38,000	172	1120	1	0.58	1200	107.14	36,000	34	31	0.5	1.47	2	6.45
90	300,000	668	400	18	2.69	600	150.00	300,000	236	46	9	3.81	4	8.69
91	300,000	640	2300	112	17.50	4300	186.96	300,000	900	145	18	2.00	7	4.83
92	36,000	191	2000	8	4.19	2470	123.50	36,000	575	53	77	13.39	31	58.49

Rodac plate has 4 sq in of surface.

*Counts at the end of 1 hr were taken as 100% to express counts at the end of 4 hr.

Table 7.--Influence of a bipolar-oriented electrical field on the deposition of aerosolized bacteria on the electrode[1] and surfaces of the chamber.

Trial No.	Electrode Surface (plastic coated)				Door Surface (aluminum)			
	No. Samples	Mean Deposition Rate ft/hr Test	Control	Difference[3] %	No. Samples	Mean Deposition Rate ft/hr Test	Control	Difference[3] %
88	8	0.02890	0.00294	10.17	4	0.29255	0.00211	0.720
89	10	0.00604	0.00150	24.79	6	7.41210	0.00063	0.009
90	10	0.01533	0.00063	4.14	3	1.07600	0.00031	0.030
91	8	0.09833	0.00084	0.85	4	2.07360	0.00038	0.020
92	9	0.04090	0.00611	14.94	5	3.96560	0.00178	0.050
105	5	0.02194	0.01445	65.86	3	3.62910	0.00123	0.030
106	7	0.05631	0.02117	37.60	4	1.40400	0.00240	0.170
107	11	0.06774	0.00431	6.14	6	1.18523	0.00094	0.080
108[2]	10	0.02205	0.00382	14.32	5	0.06163	0.00099	1.600
	Wall Surface (stainless steel)							
126[2]	6	0.22818	0.00568	2.49	6	0.45440	0.00516	1.140
127[2]	6	0.34716	0.00330	0.95	6	0.75895	0.00465	0.610

[1]Field voltage was 14,000 at 1 m from electrode.

[2]*Bacillus subtilis*, otherwise *Serratia marcescens*.

[3]$\frac{\text{Control}}{\text{Test}} \times 100$

The greatest attraction of airborne bacteria or spores has been observed on the aluminum door of the chamber as shown by Table 7. The viable number of microorganisms was at times as much as 100 times greater than on the charged electrode which produced the field voltage. The difference in percentage deposition among trials was caused by the variation in airborne concentration of microorganisms. However, the higher the number of microorganisms, the less the difference in deposition between the charged and uncharged electrodes tended to be. Results of two trials shown in Table 7 seemed to indicate that there was a slightly greater deposition of spores of B. subtilis on the aluminum door than on the stainless steel wall of the chamber when the bipolar-oriented electrical field was present. However, the difference between the door and walls does not approach the difference between the door and the electrode.

In order to evaluate deposition of airborne B. subtilis in the metal chambers after aerosolization of high concentrations of spores and exposure to the bipolar-oriented electrical field for 8 hr, the spore concentrations were determined at various locations as shown in

Table 8. The following observations can be made on the basis of these results (Table 8): 1) the deposition of B. subtilis spores under the influence of the bipolar-oriented electrical field was much greater on the door and wall surfaces of the chamber with the charged electrode (29.1 to 605.5 times higher) than in the control chamber; 2) the deposition on the charged electrode was also considerable (51.2 times that of the uncharged electrode); 3) the least increase in deposition was observed on the floor (average 1.5 times greater); 4) moderate increase of deposition was on the ceiling of the test chamber (15.8 times greater); and 5) in the test chamber the deposition was greater along the length of the walls, door and floor midway between the two edges than at 6 in distance from the edges.

The explanations for the above observations in order are as follows: 1) the majority of the airborne B. subtilis spores were deposited on metal surfaces under the influence of the field perhaps because the spores were charged similarly to the electrode or oppositely to the metal surfaces; 2) however, some of the spores may bear the opposite charge to the electrode, consequently,

Table 8.--Influence of the bipolar-oriented electrical field on deposition of airborne Bacillus subtilis spores on metal surfaces of the chambers.

Surfaces sampled	Number of locations	Count* in the chamber		Times increase compared to control
		with uncharged electrode	with charged electrode	
Ceiling	10	5	79	15.8
Wall I				
Sec. A	4	10	1964	196.4
Sec. B	6	16	817	51.0
Avg.	10	13	1267	97.5
Wall II				
Sec. A	4	4	2422	605.5
Sec. B	6	6	867	144.5
Avg.	10	8	1488	186.0
Wall III				
Sec. A	4	13	1286	98.9
Sec. B	6	24	833	34.7
Avg.	10	20	1014	50.7
Wall IV				
Sec. B	6	25	1193	47.7
Aluminum door				
Sec. A	4	124	4052	32.7
Sec. B	6	96	2798	29.1
Avg.	10	107	3299	30.8
Floor				
Sec. A	4	3613	6111	1.7
Sec. B	6	2283	2834	1.2
Avg.	10	2814	4145	1.5
Electrode (plastic coated)	3	28	1434	51.2

*No. of B. subtilis spores deposited per 4 sq in (Rodac plate).
A Length of the wall, door, or floor midway between the edges.
B Length of the wall, door, or floor 6 in from the edges.

they are attracted to and retained on the electrode; 3) the relatively small increase on the floor is probably due to the fact that under the influence of gravity in both chambers considerable settling occurred; 4) the ceiling being plastic did not attract bacterial spores to the same extent as the metal surfaces, which may have had a charge opposite to that of the spores; 5) more spores were deposited on metal surfaces which were nearer to the electrode. This is indicated by Figure 21. Where the electrode was 1 ft from the floor (Figure 21, Location 2), the deposition was the greatest, compared to the other locations on the floor which were each 1 ft farther away.

From the trials carried out by the bipolar-oriented electrical field it is apparent that the reduction of populations of airborne microorganisms in the enclosed chamber was mainly due to their removal from the air rather than a lethal effect on viability, although some effect on viability should not be ruled out. The different behavior of the various organisms tested (vegetative cells, bacterial spores, and mold spores) may be interpreted by their carrying different amounts of

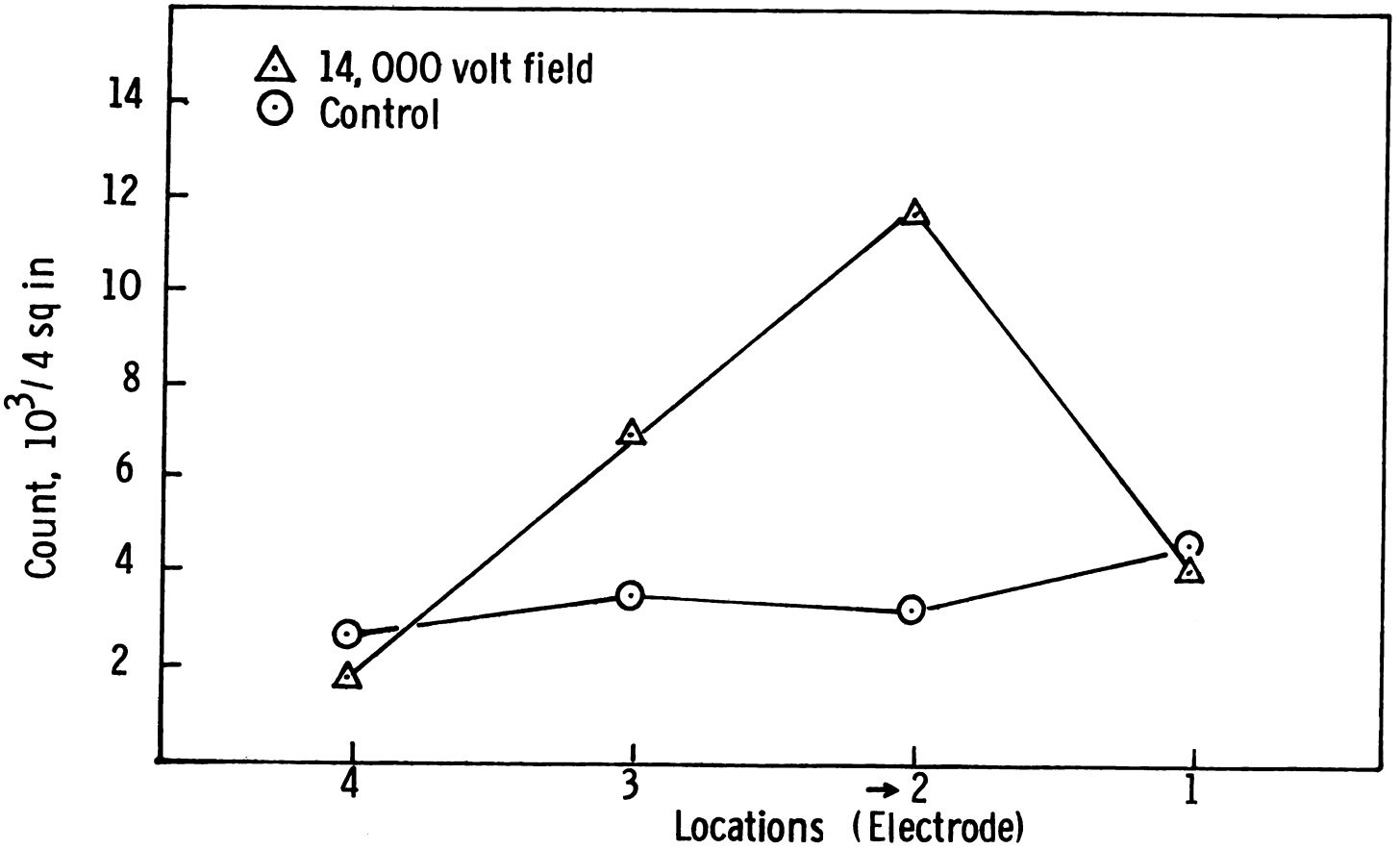

Figure 21. Distribution of Bacillus subtilis spore deposition on the stainless steel chamber floor under the influence of the bipolar electrical field. Locations are 1 ft apart.

electrical charges. Another interpretation could be that they differ in their viability under the influence of the electrical field. However, in any operation, such as aseptic packaging, where the reduction of populations of airborne microorganisms is critical, more rapid removal is necessary than the above tests indicated was possible with the bipolar-oriented electrical field. Therefore, further improvement in the design or further application of the design is necessary before it can provide a practical means of control of airborne microorganisms in food processing plants.

SUMMARY AND CONCLUSION

In summary the results are:

1. Broken survival curves of S. newbrunswick, aerosolized from distilled water, indicated that the resistant airborne bacteria may survive for at least several hours.

2. Aerosolizing from skim milk as compared to distilled water increased D values of airborne S. newbrunswick, and the protective effect of skim milk increased as the relative humidity of the atmosphere decreased.

3. Change in air temperature from 10 to 21 C produced moderate increase in the initial death rates of S. newbrunswick aerosolized in distilled water at RH values of 30, 50, 70, and 90%. The death rates increased with decrease in RH at both temperatures.

4. Gradual or rapid rehydration of the airborne bacteria captured on filters did not show significant difference in the release of potassium, sodium, magnesium, phosphates, amino acids, simple carbohydrates, or protein-like substances.

5. Gradual rehydration of the organisms on the filter membranes at various RH levels resulted in a weight gain characterized by sigmoid curves as the RH was increased to saturation.

6. Nearly tenfold increases in the amount of 265 nm light absorbing material were observed in the supernates of airborne S. newbrunswick as compared to the unaerosolized control. Chromatographic separation of the 265 nm absorbing material on Biogel 2 yielded a peak with similar elution volume to that of adenosine monophosphate.

7. Electron microscopic findings indicate that airborne and freeze-dried cells respond similarly to embedding and fixing procedures whereas the unaerosolized control had undergone plasmolysis.

The aerosolized, rehydrated, and freeze-dried cells showed a depletion in ribosomal granules concomitant with the appearance of 265 nm absorbing material in the supernatant fluid of these cells.

8. The bipolar-oriented electrical field when operated at 6,000, 10,000, 14,000, and 20,000 volts reduced various species of airborne microorganisms as the voltage was increased. The reducing effect leveled off between 14,000 and 20,000 volts.

9. The bipolar-oriented electrical field reduced airborne P. roqueforti by an average of 37.5%, S. marcescens 43%, P. fragi 59%, C. lipolytica 48%, and B. subtilis spores 52%. Under the influence of the field the death rate of S. newbrunswick increased by 36% but there was no significant change in the death rate of S. marcescens. Most of the reduction of the airborne microorganisms under the influence of the field was due to their removal from air by deposition on metallic surfaces of the walls or the floor of the experimental chamber.

In conclusion, death rate of airborne S. newbrunswick was slow enough to permit some survival at least for several hours. The effect of the bipolar-oriented electrical field on airborne microorganisms, including salmonellae, was not sufficient to provide an effective means of control of airborne contamination.

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LITERATURE CITED

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APPENDIX

APPENDIX

Preparation of Epon 100% embedding material*

Mixture A

Dodecyl Succinic Anhydride (DDSA) 80 g, add Epon 812 100 g on top of DDSA, and shake until homogeneous.

Mixture B

Nadic Methyl Anhydride (NMA) 85 g, add Epon 812 100 g on top of NMA, and shake until homogeneous.

Epon 100%

Mix thoroughly 5 ml mixture A with 5 ml Mixture B adding 4 drops of accelerator DMP-30, tri-dimethylaminomethyl-phenol.

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*Embedding materials obtained from and prepared by instructions of Ladd Research Industries, Inc. Burlington, Vermont.