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CIVIL ENGINEERING STUDIES

SANITARY ENGINEERING SERIES NO. 21

STUDIES ON THE VIABILITY OF AIRBORNE BACTERIA

By
CALVIN P. C. POON

Supported by
DIVISION OF OCCUPATIONAL HEALTH
U. S. PUBLIC HEALTH SERVICE
RESEARCH PROJECT OH-00127

DEPARTMENT OF CIVIL ENGINEERING
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
MAY, 1964

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by

CALVIN PO CHUEN POON

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Department of Civil Engineering

University of Illinois

Urbana, Illinois

May, 1964

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STUDIES ON THE VIABILITY OF AIRBORNE BACTERIA

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Department of Civil Engineering

University of Illinois, 1964

It is beyond doubt that the transmission of respiratory diseases is brought about by airborne microorganisms. In order to understand how airborne microorganisms survive in the air, the factors affecting their viability must be delineated. Such information will assist in establishing more effective methods of air disinfection. The present work involved the study of airborne Escherichia coli in two phases, namely, the investigation of the factors which had immediate as well as those with long term effects on their viability, and the investigation of the conditions under which "inactivated" airborne E. coli could be reactivated.

To study the significance of various factors on the viability of airborne E. coli, two storage chambers were constructed. One of them provided a storage time from one-half second to four and one-half seconds while the other one provided a storage period of several hours. Using these storage chambers, it was possible to study the immediate effects as well as the long term effects.

It was observed that relative humidity (R. H.) and temperature together had an instantaneous effect on the airborne cells by evaporating protein bonded water from within cells. The death rate increased in direct proportion to the decrease of R. H., but increased exponentially as the temperature increased. The evaporation of water droplets expressed by Fuch's equation gave identical results. The rate of death of airborne E. coli was found to be directly proportional to the rate of water evaporation. In comparison to the case of E. coli aerosols sprayed from distilled water, the presence of sodium chloride in the aerosol tended to increase the rate of death at low temperature

and high R. H. levels because of its immediate dehydration effect on the cells. At high temperature and low R. H. levels, however, sodium chloride tended to protect the cells from rapid water evaporation and consequently gave a lower rate of death. Because of its hygroscopic property, glycerol at low concentration reduced the rate of water evaporation and therefore the rate of death of airborne cells.

Airborne E. coli were found to be able to synthesize metabolites at room temperature and R. H. The rate of death of E. coli during a long storage period, as determined by comparing the number of living bacteria before and after storage, was lower because of the synthesis of new cells. Active cells, harvested in accelerating growth phase, showed a lower death rate than that of non-active cells, harvested in stationary growth phase, because the former readily utilized metabolites for growth and multiplication.

In the presence of several intermediate metabolites involved in the Krebs cycle reactions, airborne E. coli cells inactivated by "desiccation" were found partially capable of resuming multiplication at room temperature and R. H. It was believed that the reactions in the Krebs cycle were blocked in the process of desiccation. The enzymes involved in the production of aspartic acid and glutamic acid were inactivated. By supplying precursory metabolites such as citric, α -ketoglutaric and oxaloacetic acids, the cyclic reactions were restored as well as the ability of the cells to multiply. The degree of disinfection of air should therefore be raised to such an extent that metabolic recovery would fail.

ACKNOWLEDGEMENTS

Much of the data in this report is taken from the experimental works supported by the National Institute of Health, U. S. Public Health Service, Research Project USPH OH-00127. The author wishes to take this opportunity to express his gratitude for being authorized to use all this data in presentation.

The author also wishes to thank particularly the following persons:

Dr. I. Hayakawa, Assistant Professor of the Department of Civil Engineering, for his supervision of the experimental works, his valuable comments, advice and suggestions as well as his kindness in allowing the author to use his equipment and space in the Air Pollution Laboratory, without which the author's work could not have been satisfactorily accomplished.

Professor R. S. Engelbrecht, of the Department of Civil Engineering, not only for offering advice and assistance in the author's experimental works and taking his valuable time to read over the paragraphs while this report was being prepared, but also for his constant advice on the academic as well as personal matters during the author's course of study at the University of Illinois.

Professor B. B. Ewing, of the Department of Civil Engineering, for his advice and assistance in the course of the author's work, particularly in purchasing and handling radioactive material.

The author is indebted to Mr. N. M. Norton who helped in constructing much equipment necessary for the experimental works, as well as to all Sanitary Engineering Laboratory personnel who have helped the author in many ways which led to the completion of his work.

This report is a partial fulfillment of the requirements of the degree of Doctor of Philosophy in Sanitary Engineering, Department of Civil Engineering, University of Illinois, Urbana, Illinois.

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I. INTRODUCTION

A. Airborne Infection

The belief that disease may be airborne is centuries old, but only since the growth of modern microbiological science has the medical significance of bacterial aerosols become clear. In recent decades epidemiological investigations of respiratory diseases such as pulmonary tuberculosis, influenza and the common cold, have established beyond doubt the dissemination of disease by airborne microorganisms and methods of air hygiene such as disinfection by ultraviolet light and by chemical agents have been applied increasingly to their control.

Various bacteria, viruses and molds represent the types of organisms most commonly involved in airborne infection. A typical bacterial cell is 1 to 2 μ in diameter while virus particles are all smaller than 1 μ with some as small as 0.01 μ ; the size of mold spores ranges from 3 μ to 50 μ or more.

One of the most important methods of spreading certain microbial disease of man is by the expulsion of germ-laden droplets of fluid matter from the human respiratory tract. In sneezing, coughing, and even in talking, great numbers of droplets, mostly between 1 and 100 μ in diameter, of saliva and other secretions, some containing microorganisms, are expelled into the air with considerable velocities (1). The larger droplets are deposited on nearby objects or fall to the ground before they can dry, but subsequently they evaporate and their residue may be lifted again into the air, as dust, by air currents and mechanical action. Smaller droplets evaporate before reaching the ground, thus leaving any microorganisms suspended in the air, called "droplet-nuclei." Such "droplet-nuclei" may remain airborne for long periods of time and be carried long distances.

Another method of spreading microbial disease of man is the indirect liberation of microorganisms into the air from secretions that have dried on handkerchiefs or bedclothes. Many investigators have found that blankets

and bedding can become highly infective, and air sampling in hospital wards has shown that great numbers of bacteria are dispersed from blankets, etc., during bedmaking. The effect may be equally serious in domestic houses when a member of a family is infected with a respiratory disease, e.g., tuberculosis or influenza.

It is therefore evident that the mechanical transmission of airborne infection depends entirely on the ability of the bacteria to survive in the air. In order to understand the problem of airborne infection, a study of the viability of airborne bacteria seems promising. The inconsistent results of the research work to date also add to the importance and urgent need for further study.

B. Factors Governing the Survival of Airborne Microorganisms

Factors governing the viability of airborne bacteria are numerous. Among them are temperature, relative humidity (R.H.), particle size, the presence of toxic material, salts and growth medium. Studies in this area have been conducted over the past two decades. The effect of relative humidity and temperature on airborne cells has formed the greater part of these studies, but conclusions drawn from these studies differ widely. Some researchers have stated that the death of the airborne cell is greatest at high R.H. (4, 5), while others have stated that the greatest death occurs at intermediate R.H. levels (6, 7, 8). Some have reported, on the other hand, that the lowest R.H. levels are most lethal (9, 10). More recently, a modern technique of using radioactive cells in aerobiological studies has provided a means to distinguish between real and apparent death due to loss of viability of the airborne cells and particulate settling (10, 11). Besides, all experiments which were reported by these researchers were conducted with different rates of flow in storage chambers and consequently environmental conditions were different. The situation is further complicated when airborne cells of

different growth phases are employed for study. Another variable concerns the nature of the suspension medium used for bacterial aerosol atomization. It is obvious that any attempt to compare the data among the workers would be meaningless if all existing factors were not taken into consideration.

In order to eliminate the complicated situation, experiments in this present study were so designed that many factors were investigated separately.

C. Organisms Investigated

The choice of suitable bacteria for the study of airborne bacteria is largely dictated by the problem to be investigated. For practical purposes, pathogenic organisms involved in airborne infection are most appropriate for use. However, due to the lack of facilities in the laboratory for handling pathogens, it was decided to use non-pathogenic bacteria in the present study so as to eliminate any possible health hazard to the laboratory personnel.

Escherichia coli was chosen for the present study. This organism has been used widely by research workers studying its viability. Old findings therefore can support in the interpretation of the experiments and permit a comparison of results.

E. coli has the ability to grow in a chemically defined medium. Its growth is reasonably rapid, and it is easy to control. Another virtue of E. coli which makes it more convenient for study is that its membrane is sufficiently permeable (12). The metabolically active centers of the cells are in intimate contact with the environment. This permits rapid growth of E. coli cultures and pronounced effects on viability by the environment.

D. Purpose of the Study

It is the purpose of this study to use E. coli to investigate the effect of different factors on the viability of airborne bacteria, and, if

death of the bacteria occurs, to determine the mechanism involved. Radioactive p^{32} was used to label the bacteria in order to differentiate the physical loss of bacteria in the storage chamber or confined environment from the actual death of the organisms.

The present study was divided into two phases:

1. The first phase utilized a short storage chamber to provide storage times ranging from one-half second to four and a half seconds. This equipment was used primarily for investigating the immediate effects of temperature and R.H. as well as the general characteristics of the bacterial suspension on the death of airborne bacteria.

2. There are also factors which govern the death of airborne bacteria which do not exert an immediate effect. The second phase of the investigation was executed in order to study the long term effect of such factors as the presence or absence of growth supporting medium in the bacterial aerosols and the phases of growth of the bacteria when used in an aerosol. A long storage chamber with storage time from minutes to a few hours was provided for this purpose.

It is not the purpose of this study, however, to investigate different kinds of bacteria and to compare the results, but rather to concentrate the study on one single bacterial species. The results, therefore, cannot be applied to all the different kinds of bacteria which might be found in the atmosphere. However, the general significance of the effects on airborne bacteria under different environmental conditions will be determined.

II. HISTORICAL REVIEW

A. Studies on Droplet Infection

Accepting the facts that the human being is the recipient of infection and that the control of disease of man is the principal point at issue, diseases and their transmission may be classified generally as follows:

1. Diseases of lower animals transmitted directly to man (rabies, tularemia, glanders, etc.),
2. Diseases of animals or man transmitted by insect vectors (typhus fever, bubonic plague, spotted fever, malaria, etc.),
3. Diseases of animals or man transmitted indirectly by water, milk, food and inanimate objects,
4. Diseases of man transmitted directly
 - a. by infective droplets.....airborne infection (the respiratory diseases and others) and
 - b. by direct contact (the respiratory and venereal diseases in particular).

Of these modes of transmission, airborne infection is one of the most important. As early as 1933, Wells (13), as a pioneer in this field of studies, studied bacterial behavior in air. Wells and his associates shed new light on the mechanism of transmission of disease, especially respiratory disease, from man to man by experimental work involving droplet infection (14). They considered that under the usual conditions of humidity, particles or droplets smaller than 0.1 mm in diameter would evaporate completely before reaching the ground, leaving suspended nuclei consisting essentially of organic matter, salts and bacteria, to become, for all practical purposes, a part of the atmosphere. The survival of pathogenic microorganisms in such nuclei is, as suggested by Wells, largely a matter of their resistance to drying.

Wells' (13) postulation was entirely correct, for it has been shown that some of the respiratory pathogens, including viruses such as that of

influenza, may remain viable and infective for many hours (15). The study of viability of airborne bacteria became important. Since then, many factors governing their viability have been investigated (16, 17, 18, 19, 20, 21, 22, 23). For the past three decades, there has been a growing appreciation of the effect of temperature and humidity on the survival of airborne organisms. Unfortunately, the results to date have been so different and in fact are so contradictory that present knowledge neither gives us a logical conclusion nor leads us to an understanding of the mechanism involved in the death of airborne organisms.

B. Effect of Humidity on Viability

Early in 1935, Wells (16) suggested that the death rate of E. coli increased as the humidity approached saturation. Curves plotted from the data furnished by Wells and Riley (17) showed that the 50 percent survival time of E. coli atomized from broth was decreased from approximately 24 to 10 minutes when the R.H. was increased from 41 to 89 percent.

Williamson and Gates (18) dispersed several species of organisms, Serratia marcescens, Escherichia coli, Staphylococcus albus, Staphylococcus aureus, and Streptococcus salivarius, from distilled water into a room and demonstrated that the death rate increased as the R.H. was raised from 32 to 74 percent. Edward, Elford and Laidlaw (19) noticed that the recovery of influenza virus decreased at high humidities but attributed the loss to increased settling.

The fact that the increased settling might account for the increased disappearance of aerosols was not yet recognized in those days among most workers in this field. Therefore, Loosli et al. (20) reported that the survival time of influenza virus decreased as the R.H. increased. DeOme (4) reported Salmonella pullarum's rate of death, atomized from an aqueous suspension, increased steadily with an increase in R.H. from 15 to 80 percent.

However, they failed to notice that with increasing R.H., the coagulation of aerosols also increased. With greater coagulation, sedimentation increased rapidly, and the settling loss, but not the true death, therefore was largely attributed to the disappearance of viable cells. It was because of this fact that their results were misleading and incorrectly interpreted.

The very same reason also explained why Dunklin and Pick (6) reported that at intermediate levels of R.H. the death rate was the highest for many organisms. They studied the viability of Pneumococcus, type 1, and found a high mortality rate in the vicinity of 50 percent R.H., with lower and higher R.H. values having less effect. The same effect was found on hemolytic Streptococcus group C and Staphylococcus, although the mortality rates were smaller than that of the Pneumococcus. They concluded that at intermediate levels of R.H., the microorganisms became much more sensitive to toxic agents than at either higher or lower R.H. levels. In their studies on the effect of settling, they stated that the disappearance of microorganisms from the air was a true lethal process, rather than a manifestation of an aerosol collision process (or settling loss). However, in a figure in which they presented their data, it may be seen that the greatest settling loss (about 40 percent) occurred at 50 percent R.H. level. It is evident that the settling loss accounted for a major portion of the disappearance of microorganisms, and their assumption of no manifestation of settling loss was not correct.

Furthermore, the phenomenon of highest mortality rate at intermediate R.H. levels reported by Dunklin and Pick (6) only occurred when bacterial aerosols were sprayed from solutions containing sodium chloride. Because of this fact, their findings do not have general significance without considering the nature of the suspending medium.

There are workers who have reported highest mortality of airborne organisms at low levels of R.H. Wells (9) showed that the rate of death of airborne bacteria at low R.H. was much greater than at 50 percent R.H. The

rapid dehydration, as suggested by Wells, might contribute to the rapid death of airborne microorganisms. The rate of evaporation of small droplets of water in unsaturated air is so great that it would seem perfectly logical to correlate it with the rapid death of bacterial cells. A study, evidently stimulated by this idea, was performed by Webb (10) from which he proposed that a breakdown of a protein-water bond was the cause for death of bacteria when they were aerosolized from distilled water suspensions. However, Webb (10) did not study the common factors which affected both the rate of water evaporation and the rate of death of airborne bacteria.

C. Effect of Other Factors

In past investigations, temperature has been considered along with R. H. in all studies with airborne bacteria. As pointed out by Davis and Bateman (21), such results are generally complicated by extrinsic solutes, i.e., foreign material in the bacterial suspension from which bacterial aerosols are atomized. This means that different suspending media used in generating bacterial aerosols have a remarkable effect on the death rate of airborne bacteria. A comprehensive study has been reported by Kethley, Cown and Fincher (22) in which they revealed the significance of evaluating the nature and composition of the suspending aerosols. In their study, distilled water, beef extract broth, glycerol and a combination of all these materials were employed. It is interesting to note from their data that the aerosols, sprayed from different solutions, were very different in nature. Therefore it is easily understood why they emphasized the importance of a study of the nature of the suspending aerosols in order to properly evaluate the factors affecting the viability of airborne bacteria. A term "immediate environment" was suggested by Kethley et al. (23) as referring to the bacterial aerosol surrounded by a layer of non-living material when they are subjected to various suspensions before aerosolization. This "immediate environment" is important in all bacterial

aerosol studies, whether they are made to determine the effect of temperature and R.H. or whether they are concerned with the possible effectiveness of aerial disinfectants.

D. Physical Loss and Viable Decay

Bacterial aerosols in a storage chamber are subjected to viable decay as well as to physical loss. A failure to differentiate these two factors could lead to a significant error. In fact it has been mentioned previously that if physical loss in a storage system is neglected, analysis of the data often results in incorrect interpretations, giving a completely erroneous picture of how humidities affect the viability of airborne bacteria. This error was not recognized until Edward, Elford and Laidlaw (19) showed that the greater disappearance of influenza virus at high humidities was due to increased settling. It was Henderson (24) who first reported a technique to differentiate losses due to trauma from those of mechanical origin, i.e., any deposition of aerosols during passage from the spray atomizer to the sampling point.

Since Henderson's study, a variety of techniques have been developed to detect physical loss. Henderson confirmed that physical losses can be assessed separately by spraying a suitable chemical substance in solution and then measuring the amount collected per unit volume of flow. The results so obtained were expressed in percentage of recovery of the amount of substance sprayed. He referred to this ratio as the absolute spray factor (A.S.F.). In estimating the A.S.F., operating conditions should be kept constant, and a stable indicator substance should be used. The dye, sky blue F.F. (color index No. 518), was found satisfactory for this purpose. However, any material such as a dye which has a different physical characteristic from that of the bacterial cells would not settle at the same rate as the bacterial aerosols and, consequently, the physical losses would not be the same.

Another method for detecting physical loss was to expose agar plates at the bottom of the storage chamber to determine the bacterial settling. The settling samples were taken with respect to time so that the rate of fallout under the experimental condition could be calculated, according to Kethley, Fincher and Cown (25).

Dunklin and Pick (6), instead of measuring the number of organisms, dyed the bacteria in the sprayed solution and measured the color in the collected sample by using a spectrophotometer. The difference of the color intensity was then correlated with the number of organisms lost due to deposition.

All the methods described so far depend entirely on the efficiency of sampling, like any other analytical technique. This limits their use in most works.

Two other methods which eliminate the disadvantage of poor efficiency of sampling have been proposed. One was described by Dimmick (26) in which a recording light-scattering photometer and electronic particle counter were attached to the storage chamber. This technique employed an improved right-angle optical system and a single electron multiplier phototube. O'Konski and Doyle (27) also used a counter photometer in their study of aerosols. The bacterial aerosol behavior is not disturbed since samples are not removed from the aerosol chamber. This light scatter decay method yields data in terms of rates, and although some of the rates are approximations, a sensitive measure of aerosol presence and behavior is continuously available. The calculation of the physical decay curve requires only a relatively short time. However, it is not applicable to small aerosol chambers where the storage time is extremely short.

Another method developed only recently, is to label bacterial cells with radioactive phosphorous, P^{32} (10, 11). Dead tracer cells labelled with P^{32} are mixed with living non-labelled cells under study in the same suspension.

The radioactive tracer cells are subjected only to physical loss in the chamber. Therefore the percentage of recovery of these radioactive cells permits calculation of the physical loss in the system. The loss of non-radioactive living cells under investigation would suffer the same physical loss provided that both labelled cells and non-labelled cells were thoroughly mixed in the system and their physical characteristics were not different. Theoretically, this tracer method is more direct than the light scatter decay method. Because it yields absolute values, it can be employed to determine recovery percentages and collection efficiencies upon initial aerosolization. However, this method is relatively expensive in terms of time, both in the sense of man-hours and of the waiting period before physical behavior data become available for analysis. Details of the technique involved in this method can be found in the works of Webb (10) and Harper et al. (11).

III. THEORETICAL CONSIDERATIONS

A. The Effect of Relative Humidity on the Viability of Airborne Bacteria

It has been confirmed that the persistence of airborne bacteria depends on their ability to survive at different relative humidity and temperature levels. Also, it was found recently by research workers that the lethal process is much more rapid at low R.H. levels than at higher levels.

When a bacterial culture is suspended in distilled water as the spraying solution, each aerosol particle thus formed contains an individual bacterial cell or cells surrounded by a layer of pure water. Since there is no foreign material in the droplet, no extrinsic effect is expected on the viability of the bacterial cell(s), except those associated with the temperature and R.H. in the storage chamber.

The question has been raised concerning the osmotic pressure effect on the bacterial aerosols. The osmotic pressure is changed when bacterial cells are transferred from the culture medium to the distilled water. In other words, they are exposed to a hypotonic solution after the transfer. In general, bacterial cells suspended in distilled water do not survive more than a few hours, although spores will survive for many weeks. Death of the organisms results from a variety of factors, one or more of which has been studied in most of the reported experiments. Water from a metal still, for example, often contains sufficient traces of metal ions to be toxic, and water sterilized in soda or "soft" glass contains alkali dissolved from the glass. Water freshly distilled from hard glass is neutral, but upon standing absorbs carbon dioxide from the air and becomes acid. pH can influence the survival of bacterial cells in water. Other factors, such as the number of bacteria suspended in a given amount of water, dissolved oxygen and accessibility to oxygen, etc., are likewise known to affect the survival of these organisms. The osmotic pressure of distilled water, which might be thought of as having considerable significance, is not an important factor because bacteria are

remarkably resistant to changes in osmotic pressure and are not disturbed or plasmolyzed in hypotonic or hypertonic solutions. For example, Aerobacter aerogenes can grow in media with salt concentrations ranging from less than 0.1 percent to about 12 percent. In fact, except for halophilic organisms and animal cells such as red blood cells, bacteria are relatively insensitive to hypotonic solutions as a general rule.

By eliminating the effect of osmotic pressure from consideration, the only factors that could cause damage to airborne bacterial cells, sprayed from distilled water suspension, are temperature and R.H. since no extrinsic effect by foreign matter is present. If the temperature in the storage chamber is kept within a range in which the bacteria can survive, this property is eliminated in concerning the effect on the viability of cells. In this study, for example, temperatures ranging from 20° C to 50° C were investigated with 60° C being the critical temperature at which E. coli would be killed by a continuous exposure of 30 minutes.

In considering R.H., it must be remembered that humidity is closely allied to hydration and dehydration processes, or an exchange of water molecules in and out the cell membrane. Thus, R.H. and temperature, which in turn affects the R.H., could have a combined effect on the death of bacteria by influencing the water content of cells. The rate of death is governed by the rate of change of temperature and R.H. in the environment to which the bacterial cells are exposed. The greater the rate of evaporation of water from aerosol particles, the greater the rate of bacterial death is anticipated.

B. Protein Structure and Binding Water

Just how the evaporation of water influences the viability of airborne bacteria requires an understanding of the structure of protein molecules. The primary structure of a protein molecule is the peptide chain built up by L-amino acids. Although most workers believe that there is little or no

binding of water by the peptide groups, the works of Mellon et al. (28, 29, 30) have suggested that the peptide groups can participate in water binding.

Perhaps the best evidence of the presence of water bonded to protein molecules is a study reported by Fraser and Macrae (31). According to their work, investigations of the infra-red spectrum of collagen in the $2\ \mu$ region showed that hydrated collagen contains a proportion of water molecules which are preferentially oriented with respect to the polypeptide chains. They stated:

"In the spectrum, absorption band at 5150 cm^{-1} is considered attributed to sorbed water. This absorption band may be assigned to a combination of the antisymmetrical stretching and symmetrical deformation modes of the water molecules so that the associated transition moment is parallel to the line joining the two hydrogen atoms."

The preferred orientation, as suggested by Fraser and Macrae (31), was as follows:

The configuration of the polypeptide chain in collagen is believed to be such that the $C = O$ linkages are approximately normal in direction to the collagen chain axis protruding out from the triple chain molecule, and are not available for intra-molecular hydrogen bonding as mentioned by Crick and Kendrew (32). It is possible, therefore, that the oriented water molecules are linked to these groups through hydrogen bonds as shown above.

Identical to this conclusion are the comments by Linderstroem-Lang and Schellman (33) on the reversible heat denaturation of chymotrypsinogen. They suggested that water is trapped in the interior of the native protein,

forming bonds like

which on denaturation are transformed into

There is evidence, therefore, that water is bonded in native protein molecules as an integral part of the protein structure. Also, it is quite possible that water molecules are attached to the protein at various sites. The bonds at various sites have different bond strengths, some of them being weak bonds and some very strong. Water molecules also could be attached to sites vital to cellular stability in varying degrees; therefore the sites of water-protein bonds could affect the viability of bacterial cells.

C. Denature of Protein

Since water molecules have been shown to be an integral part of protein molecules, "dehydration" of the protein results in its inactivation. Proteins constitute approximately three-fourths of the dry substance of cells and therefore they are considered as quantitatively the main material of cells. The inactivation of protein molecules eventually would lead to the death of bacteria.

The removal of water molecules within cells as a cause of rapid death is not a new idea. Scott (34) in 1958 stated that the removal of the most firmly held water molecules within cells resulted in some loss of bacterial stability. Ferry, Brown and Damon (35) in 1958 also suggested that the rate of transmission of water through the cell boundary was involved in the death of bacterial aerosols.

D. Theories of Water Evaporation

The rate of evaporation of a water drop at rest is governed by the rates of transfer of water vapor and heat between its surface and the environment. The basis of the theory of evaporation of droplets in a gaseous medium was laid by Maxwell (36). He considered the simplest case which is the stationary evaporation of a spherical droplet, motionless relative to an infinite and uniform medium. In the case of a stationary evaporation, the rate of diffusion of vapor of a droplet across any spherical surface with radius ρ and concentric with the drop is constant and is expressed by the following equation:

$$I \text{ (gm/sec)} = \frac{dm}{dt} = -4 \pi \rho^2 \frac{dC}{d\rho} \cdot D \quad (1)$$

where D is the diffusion coefficient of the vapor and C its density or concentration (gm/cm^3), m being the mass of a diffusing droplet.

Integration of the above equation gives,

$$C = \frac{I}{4 \pi \rho D} + \text{constant} \quad (2)$$

Boundary conditions for this equation are:

$$C = C_{\infty} \quad \text{when } \rho = \infty$$

$$C = C_0 \quad \text{when } \rho = r$$

where C_{∞} is the concentration of vapor at infinite distance from the drop with radius r , and C_0 is the vapor concentration at the surface of the drop which is assumed to be equal to the concentration of saturated vapor at the temperature of the droplet.

From equation (2),

$$C - C_{\infty} = \frac{I}{4 \pi \rho D} \quad (3)$$

Substituting $C = C_0$, and $\rho = r$ into Equation (3)

$$\begin{aligned} I &= I_0 = 4 \pi r D (C_0 - C_\infty) \\ &= -4 \pi r D (C_\infty - C_0) \end{aligned} \quad (4)$$

This is the classical equation of water droplet evaporation developed by Maxwell (36).

Equation (4) shows that the rate of evaporation of a liquid droplet in a gaseous medium is proportional not to the surface of the drop, as in evaporation in a vacuum, but to the radius of the drop. In the case of very small droplets, however, the evaporation will be proportional to the surface of the drop, because for small drops the evaporation is identical to an evaporation into a vacuum. This matter will be discussed later since it is important in the present study where small size droplets were involved.

In reality, the evaporation of a droplet cannot be a stationary process since the radius and the rate of evaporation is constantly decreasing. As shown by Fuchs (34), when $C_0 \ll \gamma$ where γ is the density of the liquid drop, the evaporation can be regarded as quasistationary, i.e., one can assume that the rate of evaporation at a given moment is expressed by Equation (4).

If it is assumed that the vapor obeys the ideal gas laws and if its concentration is expressed as the partial vapor pressure p , then

$$C = \frac{p M}{R T}$$

where R is the universal gas constant and T the absolute temperature.

Substitute into Maxwell's equation,

$$-\frac{dm}{dt} = \frac{4 \pi r D M (P_0 - P_\infty)}{R T} \quad (5)$$

where M is the molecular weight of the evaporating liquid in its gaseous form.

If the pressure is expressed in mm Hg, and the concentration in gm/cm³, then

R is equal to $82.05 \times 760 = 62,360 \text{ cm}^3 \cdot \text{mm(Hg)/deg. mole.}$

Since the evaporation of very small droplets is proportional to the change in surface area, Equation (5) may be transformed into:

$$-\frac{dA}{dt} = \frac{8 \pi D M}{R T d} (P_0 - P_\infty) \quad (6)$$

with d being the density of the liquid drop. ($\gamma = d$)

This equation is more appropriate for use in considering small droplets and aerosols of small sizes.

Another approach to the derivation of an equation of water droplet evaporation was developed by Fuchs in 1934 and reviewed by Green and Lane (37). Fuchs considered the diffusion process as starting not directly at the surface of the evaporating sphere but at a distance of Δ apart from the droplet surface. In other words, the evaporation starts from the surface of an enveloping sphere of radius $r + \Delta$, where Δ is the thickness of the enveloping layer which is of the order of the mean free path of the diffusing molecules. Very few molecules will be present in the spherical shell of thickness Δ which represents the distance traveled by an evaporating molecule before it collides with a gas molecule, and can be calculated from the formula,

$$\Delta = \lambda \left[\frac{m_1 + m_2}{m_1} \right]^{\frac{1}{2}} \quad (7)$$

where λ is the mean free path of the evaporating molecules, m_1 is the mass of an air molecule, and m_2 is the mass of a diffusing molecule.

The rate of evaporation into a vacuum is $4 \pi r^2 \alpha C_0$ molecules per second, $4 \pi r^2$ is the surface area of the droplet, with a vapor concentration at the surface near saturation, C_0 . α is the evaporation coefficient, i.e., the fraction of the molecules striking the surface which condense, and v is a constant which is equal to $(R'T/2\pi m_2)^{\frac{1}{2}}$ with R' being the gas constant per molecule. If the vapor concentration at a distance $r + \Delta$ from the center of

drop is C_1 molecules/cm³, then molecules arriving at the surface which is $r + \Delta$ away from the center of the droplet would be evaporated at the rate of $4 \pi r^2 v \alpha (C_0 - C_1)$. At equilibrium, this should be equal to the rate at which molecules leave the surface by diffusion. Therefore

$$4 \pi r^2 v \alpha (C_0 - C_1) = 4 \pi (r + \Delta) D C_1 \quad (8)$$

where D is the diffusion coefficient in Maxwell's equation.

Rearranging the equation, it becomes:

$$C_1 [(r + \Delta) D + r^2 v \alpha] = C_0 r^2 v \alpha$$

and,

$$C_1 = \frac{C_0 r^2 v \alpha}{(r + \Delta) D + r^2 v \alpha} \quad (9)$$

From Equation (5),

$$- \frac{dm}{dt} = 4 \pi r D C_0 \quad (10)$$

which expresses the loss of mass per second at the droplet surface into the surrounding vacuum.

Substituting Equation (9) into Equation (10), with C_1 replacing C_0 and $r + \Delta$ replacing r , the loss of mass per second at the surface of the enveloping shell of thickness Δ becomes:

$$\begin{aligned} - \frac{dm}{dt} &= 4 \pi (r + \Delta) D \frac{C_0 r^2 v \alpha}{(r + \Delta) D + r^2 v \alpha} \\ &= 4 \pi D C_0 \frac{r^2 v \alpha (r + \Delta)}{(r + \Delta) D + r^2 v \alpha} \\ &= 4 \pi D C_0 \frac{r^2 v \alpha}{D + r^2 v \alpha / (r + \Delta)} \\ &= 4 \pi D r C_0 \frac{1}{D / (r v \alpha) + r / (r + \Delta)} \end{aligned} \quad (11)$$

When r is large in the case of large droplets at atmospheric pressure, Δ is comparatively very small and $r/(r + \Delta)$ approaches unity, whereas $D/r\nu\alpha$ will be negligible compared to unity. Then Equation (10) is derived. On the other hand, for very small drops, Δ is large compared to r , therefore the term $r/(r + \Delta)$ approaches zero.

Dividing both sides of Equation (11) with a unit area, the expression of the rate of evaporation per unit area becomes:

$$\begin{aligned} -\frac{1}{A} \frac{dm}{dt} &= \frac{4 \pi D C_0 r}{[D/(r\nu\alpha) + r/(r + \Delta)] 4 \pi r^2} \\ &= \frac{D C_0}{D/\nu\alpha + r^2/(r + \Delta)} \end{aligned} \quad (12)$$

When r is very small,

$$-\frac{1}{A} \frac{dm}{dt} = \nu\alpha C_0$$

and this is identical to the rate of evaporation into a vacuum as it has been defined previously. Therefore the assumption that the evaporation is proportional to the surface area of droplet is verified.

Equation (12) can be transformed to give the rate of change of surface with time similar to the form expressed in Equation (6),

$$\begin{aligned} -\frac{dA}{dt} &= \frac{8 \pi D C_0}{d} \frac{1}{D/r\nu\alpha + r/(r + \Delta)}, \\ &= \frac{8 \pi D M}{R T d} (P_0 - P_\infty) \frac{1}{D/r\nu\alpha + r/(r + \Delta)}, \\ &= \frac{8 \pi D M}{R T d} (P_0 - P_\infty) \frac{1}{D/r\nu\alpha}, \text{ when } r \text{ is very small,} \end{aligned}$$

and

$$-\frac{dA}{dt} = \frac{8 \pi M}{R T d} (P_0 - P_\infty) r\nu\alpha. \quad (13)$$

However, when water is evaporated, the heat is removed from the drop and the surface temperature on the water droplet is lowered. An equilibrium temperature, θ , will be reached and is lower than the atmospheric temperature in the system in which the water droplets are exposed.

This depression of droplet temperature ($T - \theta$) is significant for water droplet evaporation, because a few degrees change can cause a great change in the vapor pressure of the droplet surface.

The equilibrium temperature θ for an evaporating droplet was found by Johnson (46) to be:

$$\theta = \frac{L M D}{K R T} \frac{(f \cdot P_T - P_\theta)}{(D/rv\alpha + 1)} + T \quad (14)$$

where θ is the equilibrium temperature in absolute degree,

L is the latent heat of vaporization of the liquid, for water this is equal to 579.5 cal./gram,

M is the thermal conductivity of the air, 0.014 Btu/(cm²sec)(1°F/in.) or 0.0000288 g.cal/(cm²sec)(1°C/cm),

f is the relative humidity expressed as a fraction,

P_T is the vapor pressure of the liquid at ambient absolute temperature,

P is the vapor pressure of the liquid at equilibrium temperature, and

D is the diffusion coefficient which is equal to $0.21 + 0.0015T^\circ\text{C}$. (47)

At 30°C and 50 percent R.H. for example, the equilibrium temperature of a water droplet of 13 μ radius size is calculated from Equation (14) to be equal to 21.7°C (for calculation, see page 90). The following equation is therefore used for calculating the rate of water droplet evaporation:

$$\frac{dA}{dt} = \frac{8 \pi M}{RTD} r v \alpha \cdot (f \cdot P_T - P_\theta) \quad (15)$$

This is the basic equation to be considered in the present study.

The previous discussions only consider the case of evaporation of droplets at rest, or of droplets which have no relative velocity with the moving gas stream. When a droplet is in motion relative to the gaseous medium, whether the droplet is fixed with the gaseous stream passing by or moving along with the gaseous stream at a different velocity due to the turbulence of the flow, a wind factor should be applied. Frossling (38) showed that the wind factor is a function of the Reynold's number of the flow:

$$f^1 = (1 + 0.229 \operatorname{Re}^{\frac{1}{2}})$$

Kinzer and Gunn (39) showed through experiments that $f^1 = (1 + 0.22 F \operatorname{Re}^{\frac{1}{2}})$ with F being a function of Re and not a constant as suggested by Frossling.

E. Evaporation of Aerosols

To date, most investigations on droplet evaporation have been carried out using pure substances. Bacterial aerosols may consist of various substances which differ in physical, chemical and biological characteristics. Such aerosols are more complicated than droplets of pure substances. Take for instance the simplest form of a bacterial aerosol which is composed of a single bacterial cell surrounded by a layer of pure water, the rate of evaporation of water from this aerosol is certainly different from that of pure substance.

If the water surrounding a bacterium is free water which has no chemical bond or bonds of any form attached to the cell, it will be evaporated at a rate according to Equation (15). However, when all the free water is evaporated, the "naked" bacterial cell is then subjected to "dehydration" due to the evaporation of cellular water which takes place at a much different rate.

Considering the mechanism of water evaporation, it can be seen that the vapor concentration gradient is the actual driving force for evaporation, as described in Maxwell's equation (Equation 4). Since it is assumed that water vapor follows the ideal gas law, the temperature exerts an effect in

accordance with the relationship, $C = \frac{p}{R} \frac{M}{T}$. Therefore the rate of evaporation of water is governed only by the rates of transfer of water vapor and heat between its surface and the environment. In the case of a bacterial cell, the situation is complicated by two factors. First, water molecules within the cells are bonded at various sites, i.e., to protein molecules with hydrogen bonds. Although there are weak bonds as well as strong bonds of different strength, the average energy required to break down such bonds is much higher than that required to separate the water molecules from the free water. The rate at which such protein-water bonds are broken down is therefore a limiting factor regarding water evaporation. Secondly, the presence of the cell membrane reduces the rate of vapor and heat transfer from within the cell to the outside surrounding environment. The membrane increases the thickness of the imaginary boundary shell. A diffusing water molecule has to travel through the shell before it collides with a gas molecule in the gaseous medium for the completion of the evaporation of this particular water molecule. These two factors, protein-water bonds and cell membrane, are different with various bacteria in their effect on the evaporation of cellular water. However, the mechanism in water evaporation is essentially the same in water droplet as it is with cellular water except that the rate is much slower in the latter case. The equation expressing the rate of water evaporation should be applied equally well to both cases, though in the case of cellular water evaporation, a constant is added to the equation to account for the different rates of vapor and heat transfer. Therefore,

$$\frac{dA}{dt} = \text{Constant} \cdot \frac{8 \pi M}{RTd} r v \alpha \cdot (f \cdot p_T - p_\theta) \quad (16)$$

where α is a constant value for the cellular material of one particular kind of bacteria, whereas in the case of evaporation of pure water α is equal to 0.04 (45). It has been shown by Eisner, Quince and Slack (44) that a reduction

of this evaporation coefficient, α , by dispersing an insoluble agent in water to form an insoluble monolayer on sprayed aerosols, reduces water evaporation by a factor up to several hundred. The cell membrane would reduce α to a much smaller value, and consequently, water evaporation from within a cell would be greatly reduced.

As already described, the lethal process affecting bacteria is initiated when its protein-water bonds are broken and the "freed" water begins to evaporate. The time period for the surrounding water to evaporate to a reduced level so that the cellular water starts evaporating is, therefore, very important in any study on the viability of airborne cells. It was found that the water layer surrounding the bacteria evaporates toward completion within a short period of time. (See Appendix A.) This suggests that the bonded water molecules would be broken almost immediately after the aerosols are sprayed. This phenomenon agrees with the immediate lethal effect on bacteria.

F. The Effect of Salt on the Evaporation of Aerosols

Salt has the property of lowering the vapor pressure of the liquid medium in which it exists. One example is seen in ocean water where sodium chloride and the presence of other salts have lowered the vapor pressure to some extent. The lowering of the vapor pressure of water results in a lower rate of evaporation of the water, because of the smaller pressure difference existing between the water and the atmosphere. The following table, given in the Handbook of Chemistry and Physics (52), shows the lowering of vapor pressure by sodium chloride in water:

Concentration						
gm molecules/liter of H ₂ O	0.5	1.0	3.0	4.0	5.0	6.0
Reduced Vapor Pressure						
mm Hg.	12.3	25.5	80.0	111	143	176.5

Obviously, at low concentrations of sodium chloride, the effect of lowering the vapor pressure is not significant. For example, in 0.85 percent saline water, which is equivalent to 0.145 gram molecules/liter of H_2O , the reduced vapor pressure is only $(0.145/0.5) \times 12.3 \text{ mm Hg} = 3.57 \text{ mm Hg}$, a quantity which is so small that it is practically negligible. In bacterial aerosols, however, the salt plays a very important role in controlling the rate of evaporation of cellular water.

Food preservation utilizing high concentrations of sodium chloride so that bacteria cannot survive has been practiced for a long time. It is noted that when sodium chloride is added, the dehydration process occurs. Obviously osmotic pressure plays an important role here. Also, dehydration takes place possibly because the salt ions attract the polarizable water molecules to a very large extent. Also at high salt concentrations, the number of charged groups contributed by the salts is enormous compared with those of the proteins.

When bacterial aerosols are generated from sodium chloride suspension, they are composed of individual bacterial cells surrounded by a layer of sodium chloride solution. The concentration of the saline solution is at zero time equal to that in the sprayed suspension. But within a short period of time, the water is evaporated at a rapid rate toward completion, whereas the concentration of sodium chloride in the solution is increasing rapidly. Along with this concentration increase in the sodium chloride is the greater amount of cellular water being removed from the cell. Therefore, the mortality of bacterial aerosols is increased in the presence of sodium chloride. Furthermore the dehydration of the protein water will start before the saline water surrounding the cell is completely evaporated. Obviously the sodium chloride concentration is sufficiently high, before all surrounding water is evaporated, to attract the cellular water molecules.

G. The Effect of Growth Phases and the Presence of Growth Supporting Medium on the Viability of Airborne Bacteria:

A typical growth curve for bacteria may be represented as follows:

- where I.S. = initial stationary phase
 Lag. = positive acceleration phase
 Log. = logarithmic phase
 N.A. = negative acceleration phase
 Sta. = stationary phase
 A.D.R. = accelerated death rate
 L.D.R. = logarithmic death rate

A point that needs to be stressed here is that this growth curve, or better, multiplication curve, need not be the necessary course through which the bacteria must live since it may be influenced or controlled by changing various environmental factors such as food, oxygen supply, removal of toxic metabolic products, initial population, etc. As shown in this figure, the stationary phase in Curve A can be reached earlier as represented by Curve C or much later as represented by Curve B by changing these factors.

Consequently, when the growth curve is approaching the negative acceleration phase, the rate of multiplication could be kept in the logarithmic phase for a longer period by supplying more food, provided the other favorable conditions still exist.

Bacterial aerosols sprayed from growth supporting medium could continue to metabolize and multiply under favorable conditions. With bacterial cells in the logarithmic growth stage, the cells could carry on these activities immediately and multiplications would offset the number of dying organisms. Bacterial aerosols already in the stationary growth stage, in all probability, will not resume a rapid rate of metabolism and multiplication. The multiplication, if there is any, would be so small compared to the bacterial cells in logarithmic growth phase that it will not significantly offset the number of cells dying.

Evidence has been found that E. coli in the logarithmic phase of growth can maintain their population without growth (multiplication) by utilizing exogenous glucose (40). Magraw and Mallette (40) reported:

"A threshold level of glucose was observed below which turbidity did not change during short-time experiments. Repeated additions of glucose during prolonged incubation at 37°C, either increased the turbidity slowly or maintained it, depending on the amount of glucose. Plate counts to follow viability showed slow decreases for 10 days, while the unfed controls lost viability quite rapidly. From these results, it was concluded that E. coli specifically utilized exogenous glucose for maintenance, without growth."

It is felt that exogenous glucose could play the same role in maintaining E. coli in the aerosol state. Its presence could then be an important factor in affecting the viability of bacterial cells.

H. The Restoration of the Viability of Inactivated Airborne Bacteria

The phenomenon that evaporation of protein bonded water would cause inactivation of the bacterial protein molecules has been discussed. The

percentage of survival of airborne bacteria as governed by water evaporation is extraordinarily low according to Equation (16). The state of inactivation, however, may be different from the true death of airborne bacteria. Inactivation is not an absolute, but rather, a relative term referring to the fact that with abnormal conditions microorganisms may lose their function of cell division, though they may still grow with some of their basic functional activities remaining unaffected. If reactivation occurs under certain specific conditions, a higher percentage of recovery of surviving bacteria would be expected. Evidence has been found by Heinmets, Taylor and Lehman (49) that suspensions of E. coli, strain B/r, which had been sterilized by the action of heat, chlorine, ethyl alcohol, and "zephiran" chloride, contained viable cells when incubated with various metabolites of the tricarboxylic acid cycle. Some species of bacteria are resistant to desiccation and can persist in the air for long periods of time. Tubercle bacilli, for instance, may remain viable for months in dust and be carried into the air. It is very possible that some airborne bacteria are inactivated by desiccation, and upon incubation under optimum conditions, the resynthesis of enzymes occurs and their life process is reestablished. The phenomenon of the resistance of bacteria to desiccation is then not surprising if their activities are restored through proper incubation in a suitable medium. Therefore, one object of the present study is to investigate the possibility of reactivation of airborne E. coli through incubation with various metabolites.

The Krebs tricarboxylic acid cycle has been widely accepted as the major mechanism for the synthesis of aspartic acid, glutamic acid, and the group of amino acids derived from them. Products derived from the Krebs cycle are primarily:

Glutamic acid and derivatives

In protein:

Glutamic acid

Proline

Arginine

Others:

Glutamic peptide

Oxidized glutathione

Aspartic acid and derivatives

In proteins:

Aspartic acid

Threonine

Isoleucine

Methionine

Lysine

Diaminopimelic acid

Others:

Pyrimidines

If inactivation takes place in the synthesis of glutamic acid or aspartic acid, or in any of the protein products derived from these two acids, the bacterial cell is damaged. When some intermediate metabolites are present, some steps of synthesis in the Krebs cycle can be restored. The production of glutamic acid and or aspartic acid is restored and the viability of the bacterial cell is recovered.

IV. EXPERIMENTAL EQUIPMENT AND PROCEDURES

A. Short Storage Chamber

The main part of this chamber was made of a Pyrex glass tube of 2 inches inside diameter, approximately 64 inches long. This tube was rounded at both ends, one end serving as an outlet with an inside diameter of 0.39 inch (10 mm), with the other end having a 1 inch inside diameter tube extending out 1.5 inches from the chamber. A glass baffle with 12 round holes, about $\frac{3}{32}$ inch in diameter, separated the main chamber and the 1 inch size glass tube, so that when the aerosol flow was introduced into the main chamber, it would be uniformly distributed (see Figure 1). On top of the chamber were attached nine 0.39 inch diameter glass tubes, each about 2 inches long. Each of these tubes which were used for sampling was placed seven inches apart, and was attached to polyethelene tubing having a stopcock with a 2 mm straight bore. Near both ends of this main chamber were three openings for attaching No. 2 rubber stoppers through which thermometers were inserted. Directly under one of these openings in the main chamber was a glass well which could hold about 100 ml of water when filled up to the neck. Into this water well was inserted the web connected to the wet bulb thermometer. Dry bulb thermometers were inserted through the other two openings¹. The R.H. was calculated from the difference of the readings of the dry and wet bulb temperatures (Appendix B).

Another piece of glass tube of 1 inch inside diameter, B in Figure 1, was attached to the main chamber, C, while the other end was attached to a joint, A, which had a three-way opening for intercepting recirculated flow, R.H. controlled air, and aerosols from the atomizer, respectively. Pieces A, B and the main chamber, C, were joined together by polyethylene tubing of proper

1. Thermometer ASTM 38C, Titer, Sargent, -2°C to 68°C , Subdivisions of 0.2°C

- A. Four-way Glass Joint, Pyrex
- B. One Inch I.D. Connecting Tube, Pyrex
- C. Main Storage Chamber, 2" I.D., Pyrex
- D. Nine Outlets 0.39" I.D., Each Connected with Polyethylene Tubing to a Stopcock of 2 mm Straight Bore.
- E. Dry Bulb Thermometer
- F. Wet Bulb Thermometer
- G. Glass Baffle with 13 Holes of 3/32" Diameter
- H. No. 2 Rubber Stopper
- I. Water Well Containing Distilled Water
- V. Bacterial Aerosols from Atomizer

Cross Section of Glass Baffle

FIGURE 1
SHORT STORAGE CHAMBER
(Not to Scale)

size and sealed with sealent² to eliminate leakage completely. When conducting experiments, joint A was connected to an atomizer, Figure 2, and the outlet end of the main storage chamber was connected to a flowmeter, recirculation pump, bacterial filter, dehumidifier, in succession, back to the joint B, Figures 3, 4, and 5.

A Fisher laboratory flowmeter, with tapered graduated tube No. 2L-150 and a 0.25 inch stainless steel float, was used in all systems having air flows less than 38 l/min. For air flow of 43 l/min., another Fisher flowmeter was used³. Following the flowmeter was a Gelman vacuum-pressure pump, Model 13152, of the single piston positive displacement type equipped with self lubricating hard graphite piston rings and skirts. After passing through the Gelman pump, the flow in the system was divided into two parts; one part was exhausted, and the remaining portion was recirculated back into the storage chamber after passing through a control valve, a bacterial filter and a dehumidifier. The exhaust line was connected to a gas washing bottle containing a strong sodium hydroxide solution which was used to retain the R.A. cells in the air flow. This was followed immediately by a glass wool filter. The gas flow passed out from the filter into a fume hood with a stack leading to the roof of the laboratory. The bacterial filter was a Matheson 410 brass line filter⁴ with a membrane filter of 0.45 μ pore size added. It effectively filtered out all the bacterial aerosol from the gas stream so that clean air was ensured before it entered into the storage chamber again. The dehumidifier was simply a

2. RTV 102 Silicone Rubber Sealent, General Electric.

3. Flowmeter, Pyrex brand glass with hollow stopper mounted with four orifices, measuring approximately 0.25, 0.5, 1 and 2 mm in diameter. The flowmeter was mounted on a stand, filled with mercury and was calibrated with a wet test meter of Precision Scientific Company, Chicago Model 63123.

4. The Matheson Co., Inc., Joliet, Illinois.

- A. Air Inlet
- B. Reservoir for Bacterial Suspension
- C. Spraying Nozzle
- D. Rubber Stopper (Opening for Supplying Bacterial Suspension)
- E. Aerosol Outlet

FIGURE 2. DEVILBISS NEBULIZER NO. 40 (ATOMIZER)

FIGURE 3
SHORT STORAGE CHAMBER APPARATUS

- A. Four-way Glass Joint, Pyrex
- B. One Inch I.D. Connecting Tube, Pyrex
- C. Main Storage Chamber, 2" I.D., Pyrex
- D. Devillis Nebulizer No. 40 (Atomizer)
- E. Pressure Pump
- F. Dehumidifier
- G. Bacterial Filter
- H. Needle Valve for Flow Control
- I. Outlet (Exhaust) Control Valve
- J. Recirculation Pump With Pressure and Vacuum Gauges
- K. Flowmeter
- L. Heating Tape
- M. Powerstat

FIGURE 4. INLET SECTION OF THE SHORT STORAGE CHAMBER APPARATUS

FIGURE 5. OUTLET SECTION OF THE SHORT STORAGE CHAMBER APPARATUS

Lucite tube, 1.5 inches in diameter and 7 inches long filled up with silicate gel in the middle and with glass wool at both ends next to the rubber stoppers. The control valve, H, which controlled the rate of flow in the system, was a Hoke A431 1/8 inch brass needle valve. R.H. in the storage chamber was controlled by supplying wet and dry air, in different proportions. Air from the laboratory compressed air-line, when passed through a Fisher microfilter packed with silicate gel and a 0.45μ size membrane filter, produced the dry air. A portion of this dry and cleaned air went to a Devilbiss Nebulizer No. 40 for generating moist air. The total flow in the system was composed of the aerosol flow from the atomizer, the R.H. controlled air, and the recirculated dry and cleaned air.

Part B in the storage chamber, Figure 3, was interchangeable.

Another piece of Lucite tube of 7/8 inch inside diameter, 5.35 inches long, with five sampling outlets, was used with air flows less than 43 l/min. These five outlets, Figure 6, were 1.27, 1.75, 2.27, 3.08 and 4.30 inches from joint A, and when sampled corresponded to one-half second storage for the flows of 10.8, 12.7, 14.4, 17.3 and 21.6 l/min., respectively.

For temperature control, four heating tapes⁵, covered with silicone rubber, one-half inch wide, were employed to wrap the storage chamber from the outlet of the atomizer down to the outlet end of the main storage chamber. Two Sargent autotransformers⁶ were used for control. Heat was uniformly distributed and equilibrium temperature in the system was maintained with the aid of the recirculated flow.

5. Sargent S-40853-2, 210 watts, 70 volts, 6-foot length for each tape with single twist-lock connector.

6. Autotransformer S-30941, Sargent.

- A. Outlet End of Four-way Glass Joint, Pyrex
- B'. $7/8$ " I.D. Connecting Tube with Five Sampling Outlets
of 0.39" O.D., Lucite
- C. Main Storage Chamber
- G. Glass Baffle

FIGURE 6

SPECIAL CONNECTING TUBE FOR LOWER RATE OF FLOWS OTHER THAN 431/min.

B. Sampling Unit

There are two methods which may be used for sampling bacterial aerosols with a radioactive tracer. One method is to draw the sample onto an agar plate for determining the viable count while a separate sample is drawn onto membrane filter for radioactive count. One disadvantage of this method is that the aerosols may escape deposition on the agar surface which, as a result, gives a lower sampling efficiency. Another method is to draw the sample into an impinger containing collecting fluid. After sampling, the collecting fluid can be used for making a viable count as well as radioactive count. A higher sampling efficiency is expected by using the impinger method provided that the flow is low enough. Although sampling efficiency was not of much concern in the radioactive tracer technique used in the present study, impingers were used exclusively for sampling.

All glass, graduated midget impingers⁷ were used with 10 ml of 0.85 percent saline water as collecting fluid. A Gast vacuum pump⁸ was used to draw an air sample from the short storage chamber at a rate of 2.6 l/min which was controlled by a needle valve on the Matheson flowmeter⁹. This equipment was arranged in a series as shown in the following flow diagram:

- A. Impinger with 10 ml of collection fluid
- B. Matheson Flowmeter with Control Valve
- C. Bacterial Filter
- D. Gast Vacuum Pump

FIGURE 7: FLOW DIAGRAM OF THE SAMPLING UNIT

-
- 7. MSA all glass midget impinger for dust sampling.
 - 8. Gast 0211-V36 pump, Gast Manufacturing Corp., Benton Harbor, Michigan.
 - 9. Matheson Model 603, Tube A-2-15-B, Stainless steel float.

C. Aerosol Generating Unit

A Nebulizer No. 40 atomizer¹⁰, Figure 2, was used for generating bacterial aerosols, along with a Devilbiss Compressor 501 equipped with a bacterial filter attached to the inlet side of the compressor so as to ensure a clean air supply. The compressor had a screw adjustment so that it could be controlled for a desired pressure. This provided a definite rate of flow for the atomization operation. The rate of flow was found to be 4.5 l/min., at a pressure of 2.5 psig. When this atomizer unit was connected to a Precision wet test meter, it was found that it took on the average 59.5 minutes to completely spray 6 ml of bacterial solution in the atomizer. This rate of atomization, $6/59.5 = 0.101$ ml/min, was used throughout all experiments in the short storage study.

D. Bacterial Culture

A pure culture of E. coli was obtained from the Department of Microbiology, University of Illinois. A stock culture was maintained by transferring the culture to a new tube of nutrient agar every month.

A chemically defined medium was used for culturing E. coli prior to all experiments. This medium was one of the few defined media which have been employed in biosynthesis studies using E. coli (12):

NH ₄ Cl	-----	2.0 g
Na ₂ HPO ₄	-----	0.6 g
KH ₂ PO ₄	-----	0.3 g
NaCl	-----	5.0 g
Mg (as MgCl ₂)	-----	0.01 g
S (as Na ₂ SO ₄)	-----	0.026 g
Glucose	-----	1.0 g (in 100 ml dist. water)
Distilled water	-----	900 ml
P ³² O ₄ (as H ₃ PO ₄ in HCl solution)---		10 mc

10. The Devilbiss Co., Summerset, Pennsylvania.

Isotope P^{32} ($P^{32}O_4$ as H_3PO_4 in weak HCl solution) was obtained from Oak Ridge National Laboratory. Each shipment contained about 10 mc at the time of arrival and was diluted with distilled water into a 25 ml stock solution. To prepare the medium, 100 ml of the defined medium was placed in a 200 ml Erlenmeyer flask. The flask was inoculated with E. coli followed by 1 mc of $P^{32}O_4$. The culture was grown at 37°C.

Two growth curves of a typical E. coli culture, plotting time of growth against percentage of light transmittance of the culture measured at 560 mμ λ by a spectrophotometer¹¹, are shown in Figure 8. This figure indicates that the same growth curve can be reproduced provided that the initial population is within the limits in the range under investigation. It can be seen from Figure 8 that the logarithmic growth phase occurred from after 8 to 12 hours of growth, and that the culture entered the stationary growth phase after 20 hours of growth. All experiments in the short storage chamber were conducted with cultures harvested after they had reached the stationary growth phase, or, in other words, cultural suspensions for aerosol generating were made from E. coli cultures after 20 to 26 hours of growth.

E. Experimental Procedures in Short Storage Study

In initiating each experiment, an E. coli culture was harvested and washed three times by repeated centrifugation and resuspension in solution in the following steps. The culture from a 200 ml Erlenmeyer flask was transferred to 50 ml capacity centrifuge tubes. After centrifuging at 8000 rpm in a Servall centrifuge¹² for 10 minutes, the supernatant was decanted. The

11. Coleman Model 14 Universal Spectrophotometer.

12. Weston Electrical Instrument Corp., Model 1332.

FIGURE 8
GROWTH CURVE OF E. COLI
IN THE PRESENCE OF p₃₂

cells were resuspended in one of the following solutions, depending on which bacterial suspension was going to be studied in the aerosol system: distilled water; 0.85 percent saline water; 5 percent saline water or 0.375 percent glycerol solution. The same procedure was repeated three additional times. After these washings, the supernatant usually gave radioactive counts below 10 cpm per ml. It was assumed that all the radioactive P^{32} in the final bacterial suspension was retained inside the cells. The cells were resuspended in 10 ml of the appropriate solution. This final suspension was mixed thoroughly and 4 ml were placed in the atomizer so that the formation of clumps of bacteria was reduced in the spraying suspension.

One-tenth ml of this suspension was removed and serially diluted in a saline solution. The viable count was determined by the drop plate method using EMB agar. One milliliter of the same suspension was placed in a 2-inch diameter aluminum planchet with concentric rings. The R. A. count on this suspension, expressed as cpm per milliliter, was obtained by placing this planchet with the dried aliquot in a proportional counter¹³ and counting it for 10 minutes.

The atomizer was then connected to the short storage chamber and the pressure pump was turned on to start generating the bacterial aerosol. The recirculation pump and the moisture control air supply were turned on at the same time. The flow in the system was regulated by operating the needle valve. The R. H. was regulated by adjusting the hosecocks on the dry and wet air lines, while the temperature was regulated by controlling the powerstat connected to the heating tapes. After a steady state condition had been reached, constant temperature and R. H. at the desired level, air samples from the storage chamber were taken with the sampling unit from the various outlets. All samples, collected in the impingers, were serially diluted, and the viable

13. NMC internal proportional counter, Model DS-1A, Indianapolis.

count as well as the R. A. count were determined in the same manner as in the spraying suspension, zero time sample.

After all samples had been taken, the temperature and R. H. control, the compressor, and the recirculation pump were turned off. The atomizer was detached and replaced with another atomizer containing 4 ml of formaldehyde, 37 percent HCHO. This formaldehyde solution was sprayed with the recirculation pump operating for 5 minutes so as to disinfect the system. Then everything was turned off. The system was completely closed and let stand for about one hour. Recirculation was initiated again only with wet air supply so as to remove the excess formaldehyde in the system. This was necessary so that the viability of the bacterial aerosols used in the following experiment would not be affected.

The EMB agar plates were stored at 37°C for 24 hours and viable bacteria were counted with the aid of a Quebec colony counter. Planchets containing 1 ml of sample were dried in an oven and were counted in an internal proportional counter.

F. Calculation

If the number of viable cells per 0.1 ml of spray suspension is equal to V_o , the R. A. count per minute per 0.1 ml of spray suspension is $(R. A.)_o$, the ratio $\frac{V_o}{(R. A.)_o}$ represents the number of viable cells per unit radioactive count. The total cells regardless of their viability in each of the samples could be calculated as follows:

$$T_t = \frac{V_o}{(R. A.)_o} \times (R. A.)_t$$

where T_t = total cells in 0.1 ml of sample (collecting fluid) with storage time t , and

$(R. A.)_t = R. A. \text{ counts per minute per } 0.1 \text{ ml of collecting fluid.}$

Because a portion of the bacteria died off during storage time t , the total cells T_t were composed of living and dead cells. The number of viable cells per 0.1 ml of the collecting fluid can be determined by drop plate counts, as

V_t . The difference between T_t and V_t was the actual death of bacteria.

$\frac{V_t}{T_t} \times 100$ was the percentage of survival after storage time t , or $100 - \frac{V_t}{T_t} \times 100$

was the percentage of death of bacteria after storage time t .

G. Long Storage Chamber

The chamber used in the long storage studies was made of a 5.56 inch inside diameter Lucite tube, 4 feet long. A Serdex humidity indicator¹⁴, a thermometer and a manometer were attached to the chamber as indicated in Figure 9. Bacterial aerosols were sprayed from an atomizer located on the upper part of the chamber. The atomizer was operated together with a Devilbiss Compressor 501. The bacteria laden air was recirculated by a Dynapump pump¹⁵ through a 0.5 inch diameter Pyrex glass tube back into the main storage chamber at the bottom. The rate of recirculation was 3.7 l/min. The flow coming in from the bottom part of the chamber was distributed through a baffle with 23 holes of one-half inch diameter. Three sampling outlets, each of 3/8 inch diameter, were located along one side of the chamber at various elevations, 30, 10, and 3 inches from the bottom. A R. H. control line was attached to the recirculation line ahead of the pump, in order to supply dry or moist air to the storage chamber when correction of the R. H. in the storage chamber was necessary.

14. Serdex Relative Humidity Indicator Model 201, Bacharach Industrial Instrument Co., Pittsburgh, Pennsylvania.

15. Pressure-Vacuum Dynapump, Model 12-3097, Fisher Scientific Company, Chicago, Illinois.

- A. Devilbiss Compressor 50l
- B. Devilbiss Nebulizer No. 40 (Atomizer)
- C. Main Storage Chamber
- D. Serdex Relative Humidity Indicator
- E. Recirculation Line
- F. Thermometer
- G. Exhaust Line
- H. Relative Humidity Control Gas Flow
- I. Recirculation Pump
- J. Powerstat (heat control)
- K. Bacterial Filter
- L. Vacuum Pressure Pump
- M. Hosecock
- N. Flowmeter
- O. Sampling Outlets
- P. Manometer

FIGURE 9

LONG STORAGE CHAMBER APPARATUS

When a sample was being removed from the storage chamber, a clean air supply was introduced into the chamber at the same rate as the aerosol sampling so as to replenish the volume sampled. This was made possible by using a vacuum pressure pump (Gelman Model 13152) with a bacterial filter attached to the inlet side. The clean air thus obtained went through a Fisher flowmeter into the chamber with its flow rate regulated by a hosecock in accordance with the rate of sampling, 2 l/min.

The temperature control unit and the sampling unit were the same instruments used in the short storage chamber.

H. Experimental Procedures in Long Storage Study

An E. coli culture was harvested and washed three times by repeated centrifugation and resuspension in solution using the same procedures followed in the short storage study. Distilled water, 2.5 μ mole glucose solution, cultural medium, and various metabolites of the tricarboxylic acid cycle were used for washing and final preparation of the suspensions for aerosols generation.

The storage chamber was purged with cleaned dry air to reduce the R. H. down to approximately 20 percent. Temperature was adjusted to a constant value before the aerosols were introduced. When aerosols were sprayed into the chamber, the recirculation pump was turned on and the exhaust line was opened. The concentration of bacteria in the chamber increased as the R. H. increased. When the R. H. had reached the desired level, the aerosol supply was stopped and the exhaust line was closed. A sample was taken immediately as the zero time sample. Subsequent, hourly samples were taken for four hours total storage time. The settlement loss in the system with continuous recirculation was found to be very great. Therefore, the recirculation pump was

turned on every five minutes and the air circulated for 30 seconds. Through this procedure, the loss due to settlement was reduced so that sufficient bacteria were found in the last sample for both viable and radioactive counts. Due to the decreasing concentration of bacteria in the chamber air, increasing amounts of sampling volume, 2/3, 2, 3, 5, and 10 liters of air were drawn from the chamber for the 0 time, 1, 2, 3, and 4 hour samples respectively. The increasing sampled volume provided a measurable number of bacteria for both viable and R. A. counts for each sample.

E. coli aerosols sprayed from various solutions were sampled at a flow rate of 2 l/min. using impingers, each containing 5 ml of 0.85 percent sodium chloride solution. Following collection, these samples were plated immediately. In order to determine the effect of recovery of activation and growth, different buffered metabolite solutions were used instead of the isotonic saline solution. Samples taken in such metabolite solutions were plated and then incubated for 24 hours at 37°C before they were plated again on EMB agar. The 24 hour incubation period was found necessary for the inactivated cells to become activated to the metabolites and to resume their growth ability.

After all samples had been taken, the system was cleaned with a formaldehyde mist, followed by wet air, as was practiced in the short storage study.

Calculation of the rate of death was made in much the same way as in the short storage study.

V. PRELIMINARY EXPERIMENTS

A. Size of Aerosol

Sizes of aerosols sprayed from an atomizer depend on a number of factors, such as the type of atomizer used, salt concentration of the generating solution (41, 42), and jet location relative to the surface of the generating solution in the atomizer (43). In the present study, a bacterial suspension having approximately 2.0×10^9 cells per ml was prepared, and aerosols were generated with a Devilbiss Nebulizer No. 40 under 2.5 psig and 4.5 liters per minute of air flow. The average size of aerosol particles generated from this atomizer under the specified condition was determined indirectly by dividing the volume of suspension sprayed by the number of aerosol particles generated from this volume of sprayed suspension.

A simple experiment was conducted by spraying the aerosols on a clean glass slide held approximately 3 inches away from the outlet of the atomizer for a few seconds. The number of bacteria in each aerosol particle was counted under a microscope¹ and the average number of bacteria in each aerosol particle for all observations was determined. By dividing this average number with the concentration of the sprayed suspension, the volume of an average aerosol particle could be calculated. The results are given in Table I.

As the results show, the average size of aerosols increased with the concentration of bacteria of the sprayed suspension. The increase is not of particular significance from the practical standpoint, because bacterial aerosols under natural conditions have a greater variation of sizes. The results also show that the atomizer does not yield monodispersed aerosols.

1. E. Leitz Wetzlar microscope, with magnification x430.

TABLE I

THE CALCULATION OF THE AVERAGE SIZE OF BACTERIAL AEROSOLS

	Experiment 1	Experiment 2
No. of Counts with 1 Cell in an Aerosol	20	29
2 cells	10	17
3 cells	14	12
4 cells	6	4
5 cells	8	5
6 cells	10	1
Avg. No. of Cells in an Aerosol	$\frac{20+(2 \times 10)+(3 \times 14)+(4 \times 6)+(5 \times 8)+(6 \times 10)}{20+10+14+6+8+10} = 3.03$	$\frac{29+(2 \times 17)+(3 \times 12)+(4 \times 4)+(5 \times 5)+(6 \times 1)}{29+17+12+4+5+1} = 2.15$
Conc. of Bact. Cells in Suspension	2.40×10^9 cells/ml	2.05×10^9 cells/ml
Volume of an Avg. Aerosol	$3.03/2.40 \times 10^9 = 1.26 \times 10^{-9}$ ml	$2.15/2.05 \times 10^9 = 1.05 \times 10^{-9}$ ml
Diameter of the Avg. Size of an Aerosol	13.4 μ	12.6 μ

Although the size of aerosol particles could influence the death of bacteria within the aerosols, nevertheless an average size of aerosols could be used in the study without any significant error.

B. Growth of Culture with P^{32} and without P^{32}

It seemed desirable to investigate whether there was any effect on the growth characteristic of E. coli in the presence of P^{32} at the concentration used in the present study. Figure 10 summarized the data on the growth of E. coli cultures at different P^{32} concentrations in the growth medium.

Although it appears that all cultures were growing at different rates, part of the difference could be attributed to the errors in the techniques involved. Notwithstanding the difference, all cultures entered the different growth phases almost at the same time as shown in Figure 10. It was concluded that a concentration of 1 mc of P^{32} per 100 ml of medium would not significantly change the growth characteristic of E. coli cultures insofar as this study was concerned.

C. P^{32} Leached out from Cells

On storage, P^{32} is slowly lost from the cells into the suspending fluid. The loss is greater for living cells than dead cells, probably because of the metabolism involved in living cells. When living cells are stored at 4°C , however, metabolism for all practical purposes ceases. The loss of P^{32} from cells stored under this condition should be negligible.

The loss of P^{32} from cells could introduce an error into aerosol experiments if the conditions of generation of the aerosols were such that particles were formed containing no R. A. cells. These particles being radioactive would give a count as if R. A. cells were present. A comparison of the loss of P^{32} from dead and living cells is shown in Table II.

Growth Curves of *E. coli* Culture

- ◇ Control (no P^{32})
- ▲ 0.5mc/100ml P^{32}
- 1.0mc/100ml P^{32}
- 1.5mc/100ml P^{32}

FIGURE 10

GROWTH CURVES OF *E. COLI*
IN DIFFERENT CONC. OF P^{32}

TABLE II

LOSS OF P³² FROM CELLS IN AQUEOUS SUSPENSION AT 4°C AND 25°C AS SHOWN BY INCREASED SUPERNATANT ACTIVITY.
ACTIVITY IN AQUEOUS SOLUTION SHOWN AS PERCENTAGE OF TOTAL SUSPENSION ACTIVITY.

	Storage at 4°C							Storage at 25°C				
	<u>Days</u>							<u>Hours</u>				
	0	1	2	4	6	0	2	4	6			
<u>Dead E. coli</u> (Washed 3 times with 10 percent formaldehyde)	(1)	0.14	0.90	1.3	1.5	2.1	0.14	0.21	0.28	0.33		
	(2)	0.18	0.95	1.7	1.9	2.6	0.18	0.25	----	0.37		
<u>Living E. coli</u> (Washed 3 times with 0.85 percent saline solution)	(1)	0.12	0.85	1.7	2.1	3.0	0.12	0.22	0.30	0.37		
	(2)	0.10	0.73	1.5	1.9	2.8	0.10	0.19	0.23	0.31		

Table II shows a greater loss from living E. coli cells, especially when they were stored at 25°C temperature. However, the loss was not great in that the cells could be left for days without risk of significant error, and it did not make much difference whether the cells were living or dead.

VI. EXPERIMENTAL RESULTS

A. The Rate of Death and Its Calculation in Short Storage Study

Experiments were conducted in a temperature range of 20°C to 50°C and a R. H. range of 20 to 80 percent. Facilities were not available to study temperatures below 20°C.

After aerosols were generated from the atomizer, a large number of cells were found to have died within the first half second of storage. The following 4 seconds of storage showed a much lower rate of death. The abrupt change in the rate of death was also found by Webb (10), although his findings differed in rate from the results found in these experiments. This change in death rate is shown for one of the experiments in Figure 11. All experiments for short storage gave the same results, the only difference being the slope of the line or the rate. It was observed that a straight line relationship existed from zero to 1/2 second storage time, while another straight line fitted the data for the rest of the period.

Figure 11 indicates that the death rate is proportional to the number of cells remaining alive, or

$$-\frac{dN}{dt} = k N \quad (17)$$

After integration, it becomes

$$k = \frac{1}{t} \ln \frac{N_0}{N_t} \quad (18)$$

This is the first order equation from which the rate of death, k , for any storage period can be calculated, provided that the number of cells at the beginning and at the end of the storage time, N_0 and N_t , respectively, are known.

FIGURE 11
SURVIVAL OF AIRBORNE E. COLI

The following is a typical calculation for the viability of airborne

E. coli under specified experimental conditions.

Experimental Condition:

Culture: R. A. E. coli harvested in stationary growth phase

Sprayed Suspension: Washed cells in distilled water suspension

Temperature: 30°C R. H. 50%

Rate of Air Flow: 43 l/min.

Time Sec.	¹ R.A. Count cpm/ml	Drop Plate Count cells/ml	Total Bacteria cells/ml	Percentage Survival	Percent Death
0	484,200	1.77×10^9			
1/2	253	2.52×10^5	$3.66 \times 10^3 \times 253$ $= 9.26 \times 10^5$	$\frac{2.52 \times 10^5}{9.26 \times 10^5} = 27.2\%$	72.8
1	242	2.34×10^5	$3.66 \times 10^3 \times 242$ $= 8.80 \times 10^5$	$\frac{2.34 \times 10^5}{8.80 \times 10^5} = 26.6\%$	73.4
2	228	2.10×10^5	$3.66 \times 10^3 \times 228$ $= 8.30 \times 10^5$	$\frac{2.10 \times 10^5}{8.30 \times 10^5} = 25.3\%$	74.7
3	256	2.26×10^5	$3.66 \times 10^3 \times 256$ $= 9.33 \times 10^5$	$\frac{2.26 \times 10^5}{9.33 \times 10^5} = 23.0\%$	77.0
4	253	1.83×10^5	$3.66 \times 10^3 \times 253$ $= 9.20 \times 10^5$	$\frac{1.83 \times 10^5}{9.20 \times 10^5} = 19.9\%$	78.8

The k values can be calculated for various storage times, by using equation VI.2,

$$N_o = \text{number of living cells at zero time} = \frac{1.77 \times 10^9 \text{ c/ml} \times 0.101 \text{ ml/min.}^3}{43 \text{ l/min.}}$$
$$= 4.15 \times 10^6 \text{ c/liter of air}$$

1. R. A. counts were corrected with self-absorption coefficient (Appendix C).
2. $\frac{V_o}{(R. A.)_o} = \frac{1.77 \times 10^9}{4.842 \times 10^5} = 3.66 \times 10^3$, cells per unit radioactive count.
3. $6.0/59.5 = 0.101 \text{ ml/min.}$ ---- rate of atomization (see page 39)

$$N_{1/2} = \text{number of living cells at half-second} = 27.2\% \times 4.15 \times 10^6$$

$$= 1.13 \times 10^6 \text{ c/l}$$

$$k_{1/2} = \text{rate of death for the half-second} = \frac{1}{0.5} \ln \frac{4.15 \times 10^6}{1.13 \times 10^6}$$

$$= 2 \ln 3.68 = 2.60$$

$$k_4 = \text{rate of death during the storage time from the first one-half}$$

$$\text{second to the fourth second (effective storage time} = 3.5 \text{ seconds),}$$

$$= \frac{1}{3.5} \ln \frac{27.2 \times 4.15 \times 10^6}{19.9 \times 4.15 \times 10^6} = 0.286 \times \ln 1.37$$

$$= 0.089$$

B. Summary of k Values in Short Storage Study

The k values of E. coli aerosols sprayed from distilled water suspension are shown in Table III on the following page. Correction was not made in the calculation for the natural die off of E. coli when suspended in distilled water and stored at room temperature. Since the natural decay was found to be very slow for the first 45 minutes within which time all samples would have been taken, this die off was insignificant. The decay was less than 5 percent in 45 minutes (Appendix D).

From Table III, a study of the effect of R. H. on the viability of E. coli aerosols could be made. When $k_{1/2}$ values were plotted against a function of R. H., (100 - R. H.), a family of straight lines was found. Each line represents the change of the rate of death, at a constant temperature, with respect to the change of R. H. in the aerosol system, Figure 13. The data suggest that the rate of death of E. coli aerosol increases as the R. H. in the environment decreases and a linear relationship exists. In other words, the lower the R. H., the higher would be the rate of death.

Also from Table III, a study of the effect of temperature on the viability of E. coli aerosols was made in the same manner. If $k_{1/2}$ values

TABLE III
k VALUES OF E. coli AEROSOLS SPRAYED FROM DISTILLED WATER SUSPENSION

Temp.	R. H. %	$k_{1/2}$ sec ⁻¹	k_4 sec ⁻¹	Temp.	R. H. %	$k_{1/2}$ sec ⁻¹	k_4 sec ⁻¹
20°C or 293.8°K	20	2.68	0.085	40°C or 313.8°K	20	4.75	0.165
	30	2.60	0.100		30	4.21	0.150
	40	2.46	0.075		40	3.66	0.130
	50	2.16	0.078		50	3.14	0.101
	60	1.55	0.066		60	2.88	0.092
	70	1.27	0.062		70	2.60	0.082
	80	1.04	0.062		80	2.01	0.060
30°C or 303.8°K	20	3.41	0.130	50°C or 323.8°K	20	5.87	0.185
	30	3.22	0.112		30	5.04	0.161
	40	2.82	0.102		40	4.45	0.142
	50	2.60	0.089		50	4.05	0.122
	60	2.52	0.083		60	3.30	0.116
	70	2.20	0.072		70	2.75	0.088
	80	2.00	0.060		80	-----	-----

R. A. cells in distilled water suspension
Culture harvested in stationary growth phase
Rate of air flow: 43 liters per minute

FIGURE 12
THE CHANGE OF RATE OF DEATH
WITH RELATIVE HUMIDITY AT
CONSTANT TEMPERATURE
(*E. coli* sprayed from
distilled water
suspension)

FIGURE 13

THE CHANGE OF RATE OF DEATH WITH TEMPERATURE AT CONSTANT RELATIVE HUMIDITY
(*E. coli* sprayed from distilled water suspension)

were plotted against temperature at constant R. H. levels, a family of curves was found. The change of $k_{1/2}$ values with respect to the change of temperature no longer held a straight line relationship. However, when a semilog plot was made, with $k_{1/2}$ values in logarithmic scale against temperature in arithmetic scale, Figure 13, again a linear relationship was found. This indicates that temperature affects the viability of E. coli aerosols logarithmically.

Parallel to the experiments with E. coli which were sprayed from distilled water, the same bacteria were used to carry out another set of experiments. The E. coli aerosols were generated in this case from a culture suspended in 0.85 percent saline water. The purpose was to investigate the relationship of water evaporation and the death of bacteria as influenced by the presence of sodium chloride. The $k_{1/2}$ and k_4 values found in the series of experiments are tabulated in Table IV.

As in the case of distilled water suspensions, the $k_{1/2}$ values for the E. coli aerosols sprayed from 0.85 percent saline water suspension were found to be a function of R. H. A linear relationship was found to exist (Figure 14). The rate of death increased with a decrease of R. H. Likewise an exponential function was found for the change of the rate of death with the change of temperature at constant R. H. levels (Figure 15).

Very similar results developed from these two sets of experiments using different spraying suspensions. The only difference was in the slopes of the lines (Figures 12, 13, 14, 15).

To compare the relationship between the different death rates as affected by various R. H. and temperature values with the rate of water evaporation as affected by different R. H. and temperature values, Equation (16) may be used:

$$\frac{dA}{dt} = \frac{8 \pi M}{R T d} r v \alpha (f \cdot p_T - p_\theta)$$

TABLE IV
k VALUES OF E. coli AEROSOLS SPRAYED FROM DISTILLED WATER SUSPENSION

Temp.	R. H. %	$k_{1/2}$ sec ⁻¹	k_u sec ⁻¹	Temp.	R. H. %	$k_{1/2}$ sec ⁻¹	k_u sec ⁻¹
20°C or 293.8°K	20	3.40	0.130	20°C or 313.8°K	20	4.56	0.146
	30	-----	-----		30	4.32	0.132
	40	3.10	0.093		40	3.90	0.143
	50	2.94	0.085		50	3.46	0.111
	60	2.82	0.071		60	3.20	0.100
	70	2.76	0.066		70	3.08	0.078
	80	2.80	0.058		80	2.94	0.081
30°C or 303.8°K	20	4.06	0.140	30°C or 323.8°K	20	5.10	0.162
	30	3.78	0.128		30	4.80	0.155
	40	3.44	0.121		40	4.42	0.147
	50	3.08	0.094		50	4.16	0.125
	60	2.94	0.086		60	3.82	0.122
	70	2.82	0.072		70	3.56	0.108
	80	2.86	0.070		80	-----	-----

R. A. cells in 0.85 percent saline suspension
Culture harvested in stationary growth phase
Rate of Air Flow: 43 liters per minute

FIGURE 14
THE CHANGE OF RATE OF DEATH
WITH RELATIVE HUMIDITY AT
CONSTANT TEMPERATURE
(*E. coli* sprayed from
0.85 percent saline
solution)

FIGURE 15

THE CHANGE OF RATE OF DEATH WITH TEMPERATURE AT CONSTANT RELATIVE HUMIDITY
(*E. coli* sprayed from 0.85 percent saline suspension)

In this equation, $f \cdot p_T$ is the partial vapor pressure in the environment in which the water droplet is evaporated.

When water droplets are evaporated at a constant temperature with R. H. as the variable, the previous equation becomes:

$$\frac{dA}{dt} = \text{Constant} \cdot (f \cdot p_T - p_\theta), \quad (19)$$

because v is a function of temperature only and r , for very small particles, can be considered at any instant to be a constant, in the same manner as Fuchs (36) treated very small particles in what he called the quasistationary state. For pure water, α equals 0.04 while p_T and p_θ are constant at a given ambient temperature. This equation is in the form of $y = mx + b$, a straight line function between the evaporation of a water droplet and the R. H. at constant temperature. The death of airborne E. coli and R. H. also bear the same relationship as shown in Figures 12 and 14.

On the other hand, when R. H. is held constant and temperature is varied, Equation (16) becomes,

$$\frac{dA}{dt} = \text{Constant} \cdot \frac{v}{T} (f \cdot p_T - p_\theta) \quad (20)$$

v can be expressed as $(RT/2\pi M)^{1/2}$; p_T is the vapor pressure at temperature T which can be found in the Handbook of Chemistry and Physics (52); θ and p_θ can be calculated from Equation (14). The calculations are tabulated in Table V and the relationship of water evaporation with temperature is shown in Figure 16.

Figure 16 shows that the evaporation of a water droplet changes logarithmically with that of temperature. This same relationship of the death

TABLE V
THE CALCULATION OF THE FUNCTION OF WATER EVAPORATION

$$\frac{(f \cdot p_T - p_\theta) \cdot v}{T}$$

R. H. %	Temperature abso. deg.	P _T mm Hg.	v=(RT/2πM) ^{1/2}	θ abso. deg.	P _θ mm Hg.	$f \frac{(f \cdot p_T - p_\theta) \cdot v}{T}$
20	293.8	18.427	14670	281.2	7.725	202.00
	303.8	33.321	14950	287.9	12.067	266.00
	313.8	57.740	15160	294.7	18.540	328.00
	323.8	91.570	15400	300.7	26.586	394.00
30	293.8	18.427	14670	283.4	8.969	172.00
	303.8	33.321	14950	290.7	14.450	219.20
	313.8	57.740	15160	298.1	22.791	264.30
	323.8	91.570	15400	305.2	34.482	333.50
40	293.8	18.427	14670	285.2	10.110	136.70
	303.8	33.321	14950	293.2	16.894	175.50
	313.8	57.740	15160	301.2	27.874	206.90
	323.8	91.570	15400	308.7	41.950	253.60
50	293.8	18.427	14670	287.1	11.458	112.20
	303.8	33.321	14950	295.5	19.468	144.50
	313.8	57.740	15160	304.9	32.010	157.00
	323.8	91.570	15400	311.9	49.970	199.50
60	293.8	18.427	14670	288.8	12.788	86.60
	303.8	33.321	14950	297.6	22.110	104.20
	313.8	57.740	15160	306.6	37.310	129.10
	323.8	91.570	15400	314.7	58.220	156.00
70	293.8	18.427	14670	290.3	14.084	59.25
	303.8	33.321	14950	299.5	24.767	71.15
	313.8	57.740	15160	308.8	42.175	85.00
	323.8	91.570	15400	317.2	66.510	114.80
80	293.8	18.427	14670	291.7	15.381	31.90
	303.8	33.321	14950	301.3	27.53	42.83
	313.8	57.740	15160	319.5	47.17	47.10
	323.8	91.570	15400	319.5	74.60	63.80

$-C \frac{dA}{dt}$, Rate of Water Evaporation, in Arbitrary Unit.

rate of airborne E. coli can be seen in Figures 13 and 15.

From Table V, it is also possible to determine the combined effect of R. H. and temperature on water evaporation as compared to the death rate of airborne E. coli. It is for this purpose that Figures 17 and 18 are given. These figures show the correlation of $k_{1/2}$ values and the evaporation of water under various combinations of R. H. and temperature. An arbitrary unit was chosen for the function of water evaporation, f , in plotting Figures 17 and 18.

Straight lines of good fit were found for both plots. Since small water droplets evaporate instantaneously, it is believed that the function of water evaporation plotted in these two figures truly represents water evaporation from bacterial aerosols, and that instantaneous evaporation is the factor which governs the viability of the bacteria.

Apparently the rate of death of airborne E. coli cells was in direct proportion to the rate of water evaporation from the bacterial aerosols as indicated by Figures 17 and 18. The presence of salt in the bacterial aerosols definitely affected the death rate by increasing the rate of death of bacterial cells under the same conditions of temperature and R. H., the only exception being with environments of high temperature and very low R. H. Under these conditions, the airborne bacteria died more rapidly if they were sprayed from distilled water suspension.

A question was raised, consequently, as to the mechanism whereby the salt concentration affected the viability of E. coli in the air. Since it is assumed that the presence of salt increases water evaporation by exerting its dehydration properties on the protein-water bonded within the cells, an increase of salt concentration would certainly affect the rate of dehydration and consequently the death rate of bacterial cells. Based on this assumption, an experiment was performed by spraying E. coli aerosols suspended in 5.0 percent saline water in order to see how this increase in sodium chloride

THE RELATIONSHIP OF
THE RATE OF DEATH OF AIRBORNE E. COLI TO THE RATE OF WATER EVAPORATION
(E. coli sprayed from distilled water)

FIGURE 18
 THE RELATIONSHIP OF
 THE RATE OF DEATH OF AIRBORNE *E. COLI* TO THE RATE OF WATER EVAPORATION
 (*E. coli* sprayed from 0.85% saline water)

concentration affected the death rates with respect to different environments of R. H. Table VI presents these data.

C. Viability of E. coli Sprayed from 5 Percent Saline Suspension

Table VI summarizes the results regarding the effect of high salt concentration on the viability of airborne E. coli. Correction for the natural die-off of culture was made in this case since rapid decay was found in 5 percent saline water at room temperature (Appendix D).

D. Viability of E. coli Sprayed from 0.375 Percent Glycerol Solution

In order to see whether a reduction in water evaporation would influence the rate of death of airborne bacteria, R. A. E. coli were suspended in a 0.375 percent glycerol solution for aerosol generation. Table VII indicates that the presence of glycerol, which reduces water evaporation because of its hygroscopic characteristic, decreases the rate of death to a great extent.

To compare the effects of aerosols sprayed from different suspensions on the viability of E. coli, Figure 19 was prepared. This figure provides the evidence that bacterial viability is dependent upon the extent of water evaporation from cells. With salt included in the bacterial aerosols, the organisms died at a greater rate due to the dehydration effect from the salt, and the rate increased as the concentration of salt in the sprayed suspension increased. In the presence of some hygroscopic material such as glycerol which reduced water evaporation, the death rate showed a considerable decrease as compared to bacterial aerosols sprayed from distilled water and salt suspensions.

TABLE VI

k VALUES OF E. coli SPRAYED FROM 5 PERCENT SALINE WATER

R. H. %	$k_{1/2}, \text{sec}^{-1}$	k_4, sec^{-1}
20	4.97	0.142
30	4.30	0.137
40	3.90	0.135
50	3.54	0.095
60	3.40	0.095
70	3.10	0.073
80	3.12	0.079

E. coli culture harvested in stationary growth phase

Rate of air flow: 43 l/min.

Temperature: 30°C or 303.8°K

TABLE VII

k VALUES OF E. coli SPRAYED FROM 0.375 PERCENT GLYCEROL SOLUTION

R. H. %	$k_{1/2}, \text{sec}^{-1}$	k_4, sec^{-1}
20	2.29	0.075
30	2.18	0.066
40	2.23	0.065
50	2.17	0.074
60	2.03	0.059
70	1.94	0.070
80	1.97	0.070

E. coli culture harvested in stationary growth phase

Rate of air flow: 43 l/min.

Temperature: 30°C or 303.8°K

FIGURE 19

COMPARISON OF THE RELATIONSHIP OF THE RATE OF DEATH
OF AIRBORNE E. COLI TO RELATIVE HUMIDITY FROM VARIOUS
SPRAYED SUSPENSIONS

AT
30° C AND VARYING R.H.

- ◇ — 5.00% Saline Suspension
 □ — 0.85% Saline Suspension
 △ — Distilled H₂O Suspension
 ○ — 0.375% Glycerol "

E. The Effect of Air Flow Rate on the Viability of Airborne E. coli

The factor f' (wind factor) may be included in the equation of water droplet evaporation as suggested by Frossling (38),

$$-\frac{dA}{dt} = \frac{8 \pi D M}{R T d} (P_o - P_\infty) \cdot f' \quad (21)$$

The wind factor f' equals to $(1 + K \sqrt{Re})$ and it exists only when the Reynold's number is large enough because K , a value obtained through experiments, depends on Re . The K value becomes extremely small when $Re^{1/2}$ is below unity (39).

To investigate whether the wind factor has any effect on the viability of airborne E. coli, a series of experiments were conducted at 30°C and 50 percent R. H. while the rate of air flow in the system was varied from 10.8 to 43 liters per minute. The results are summarized in the following:

<u>Air Flow Rate, l/min.</u>	<u>$k_{1/2}$, sec⁻¹</u>	<u>Standard Deviation, σ</u>
43.0	2.93	0.084
21.6	2.84	
17.3	2.70	
14.4	2.68	
12.7	2.80	
10.8	2.77	

The $k_{1/2}$ values plotted against the air flow rate for this series of experiments are shown in Figure 20. Within the range of flow rates used in this study, the rate of death was not affected to any extent. This was to be expected since the Reynold's number with all the flows investigated was less than unity with an average aerosol size of approximately 13 μ . The wind factor, as a consequence, was sufficiently small so that it may be neglected.

FIGURE 20

THE RELATIONSHIP OF
THE RATE OF DEATH OF AIRBORNE E. COLI TO THE RATE OF FLOW

F. The Death Rate of Airborne *E. coli* After the First Half Second of Storage

As previously shown, the death rate after the first half second changed abruptly and may be expressed as an exponential function with time, or first order reaction. The k_4 values reported in Tables III and IV were the death rates during storage from 1/2 to 4 seconds.

When the k_4 values are plotted with respect to R. H. at a constant temperature, or with respect to temperatures at a constant R. H., as shown in Figures 21 and 22, respectively, straight line relationships exist. Figure 23 is plotted in much the same way as Figure 17, with k_4 values against the water evaporation function, f , and smooth curves were obtained. In both curves, the same trend was found which established a definite relationship between the k_4 values and the rate of water evaporation.

More important was the fact that k_4 values increased with the function of water evaporation, though not linearly. This indicated clearly that both R. H. and temperature combined had the same effect on the viability of airborne *E. coli* during the storage period from 1/2 to 4 seconds and during the storage period of 1/2 second.

G. The Rate of Death of *E. coli* Aerosols in the Long Storage Study

These experiments were performed for the purpose of investigating the difference in the death rate of airborne bacteria from distilled water origin when stored for a longer period of time. The result of a typical experiment at 50°C and 50 percent R. H. is shown in Figure 24. When the change in the number of living bacteria with respect to time is plotted, it appears that the rate of death, defined as $k = \frac{1}{t} \ln \frac{N_0}{N_t}$, does not hold constant for the whole 4 hour period. The death rate during the first two hours of storage was calculated in this experiment to be 0.043 min.^{-1} whereas the death rate of the subsequent two hours decreased to 0.007 min.^{-1} . The same phenomenon

FIGURE 22

THE CHANGE OF RATE OF DEATH OF AIRBORNE E. COLI WITH TEMPERATURE AT CONSTANT R.H.

FIGURE 23
THE RELATIONSHIP OF THE RATE OF DEATH OF AIRBORNE *E. COLI*
TO THE RATE OF WATER EVAPORATION

Logarithmic of the Ratio of Living Cells at 0 Time and at Time of Sampling

FIGURE 24. THE CHANGE OF POPULATION OF AIRBORNE E. COLI WITH
RESPECT TO THE TIME FOR LONG STORAGE PERIOD

was observed in all experiments with E. coli aerosols sprayed from distilled water suspension. The death rate during the first two hours of storage may be expressed as k_2 and that of the subsequent two hour period as k_{2-4} . Table VIII summarizes the results found in this phase of study.

TABLE VIII
SUMMARY OF k VALUES OF E. coli AEROSOLS
DURING LONG STORAGE PERIOD

Temperature	R. H. %	k_2 , min. ⁻¹	k_{2-4} , min. ⁻¹
30°C	40	0.041	0.007
	50	0.033	0.006
	60	0.021	0.005
40°C	40	0.044	0.008
	50	0.037	0.007
	60	0.029	0.005
50°C	40	0.047	0.010
	50	0.043	0.007
	60	0.038	0.006

E. coli culture harvested in stationary growth phase
Sprayed from distilled water suspension

The change of k values, k_2 and k_{2-4} , with respect to R. H. and temperature is plotted in Figures 25 and 26 respectively. A linear relationship was found to exist for most of the data. Similar to the analysis of the short storage study, k values are plotted against the function of water evaporation, f , in Figure 27. The k_2 value increases as the value of the water evaporation function increases, but the slope gradually levels off. A linear relationship does not appear to exist. It appears that there is a certain limit for k_2 above which it is not affected by temperature and R. H. When this limit is reached, k_2 does not increase even though the temperature

Rate of Death of Airborne *E. coli*, min.^{-1}

FIGURE 27
 THE RATE OF DEATH OF AIRBORNE E. COLI TO THE RATE OF WATER
 EVAPORATION IN LONG STORAGE PERIOD
 (E. coli sprayed from distilled water suspension)

may be increased and the R. H. decreased in the environment. One may conclude that water evaporation is not the sole cause for the death of airborne E. coli under long periods of storage, and some other factors may also come into effect.

H. The Effect of Growth Supporting Medium and Growth Phase on the Viability of Airborne E. coli

Bacteria at suitable temperature and R. H. levels carry on their metabolic processes in the presence of a growth supporting medium. The growth supporting medium supplies the energy for respiration and the necessary compounds for synthesis. Without the presence of the growth supporting medium, the bacteria exist in an endogenous stage and will soon die off. The same metabolic processes can be expected with airborne bacteria provided that the equilibrium cellular water content and the moisture in the environment are capable of maintaining the biological process.

Experiments were therefore conducted using E. coli aerosols sprayed from growth supporting medium suspension. The same synthetic medium for culturing E. coli was used to wash the bacterial cells and to prepare the final suspension for spraying. Experiments were also conducted with E. coli sprayed from a glucose solution. Although glucose by itself cannot support the growth of bacteria, it was intended to see whether an exogenous energy source could help to maintain the population. All the experiments were conducted at 30°C and 50 percent R. H. Results of these experiments are presented in Table IX.

The growth phase of the bacterial culture was also taken into consideration in these experiments. Whether the metabolites of the growth supporting medium will be readily taken up by the bacterial cells depends upon the synthesis activities of the cells. These activities are considerably different during the different growth phases. From Table 9, it can be seen that in the absence of a growth supporting medium or any energy source in the

aerosols, there was no difference in their death rates even though E. coli of different growth phases were used. However, with growth supporting medium present, a very slight change in the death rate was noticed. The change occurred because the bacterial cells were able to carry on the necessary metabolic processes in an environment of 30°C and 50 percent R. H. Undoubtedly those cells which survived the desiccation were able to utilize the food material and multiplied accordingly. As a consequence, more living bacterial cells were recovered at the end of the storage period. Since bacterial cells in the accelerating growth phase are more active in their synthesis processes, they could readily take up the metabolites and grow while those at the stationary stage appeared to require a lag period before the active synthesis occurred. The results showed that the rate of death was slower in the former case than in the latter case.

TABLE IX

THE EFFECTS OF GROWTH SUPPORTING MEDIUM AND GROWTH PHASES
ON THE k VALUES OF AIRBORNE E. coli

Spraying Suspension	<u>E. coli</u> harvested in stationary growth phase		<u>E. coli</u> harvested in accelerating growth phase	
	k_2	k_{2-4}	k_2	k_{2-4}
Culture Medium	0.029	0.0053	0.027	0.0041
	0.028	0.0055	0.027	0.0048
	0.028	0.0054		
0.2% Glucose	0.031	0.0053		
	0.030	0.0055	---	---
	0.033	0.0059		
Dist. Water	0.033*	0.0060*	0.034	0.0064

*From Table VIII

NOTE: Temperature 30°C, Relative Humidity 50%

Glucose by itself can maintain the viability of bacterial cells in water suspension (40). In an airborne condition, glucose showed very little effect in maintaining the bacterial population. Although the rate of death was

on the average smaller than in the case of a control (E. coli sprayed from distilled water), the difference was not significant. Therefore no definite conclusion can be drawn that glucose can maintain bacteria cells in air.

I. The Reactivation of Airborne E. coli with Metabolites

The reactions in the Krebs cycle serve to supply intermediates for synthesis of protoplasm. The protein products of the cycle depend therefore upon these intermediates as their precursors. It is possible that by the desiccation effect, some of the reactions in the cycle are blocked and, consequently, the formation of amino acids is prevented. This leads to the inactivation of the bacterial cells. The following experiments were designed to investigate the possibility of reactivating some of the intermediate steps in the cycle. The following intermediates were used in the study:

0.2% oxaloacetic

0.2% sodium citrate

0.2% α -ketoglutaric acid

0.2% succinic acid

0.2% malic acid

All these acid solutions were prepared in 1:1 dilution with 0.1 M buffer solution ($\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$). Samples from the long storage chamber were taken with midjet impingers containing one of these metabolite solutions. It was found that in order to reactivate the cells, the collected samples should be incubated at 37°C for 24 hours. The population did not increase during this incubation period because the light transmittance of the sample solution, measured by a spectrophotometer, remained constant in this 24-hour period. The number of living cells was determined by pouring EMB agar plates before and after the incubation period. The results of a typical experiment are presented in Table X. Again here, the first order equation was used to

express the rate of death of the first two hours of storage period as well as the subsequent two hours (the rate of death here is defined as the true death which cannot be reactivated with intermediate metabolites), Figure 28.

TABLE X

E. coli REACTIVATION WITH 0.2 PERCENT BUFFERED OXALOACETIC ACID

	Time hours	Concentration of Bacterial Cells in Sampling Solution No./ml.	Percentage of Surviving Bacteria	Rate of Death min. ⁻¹	
				k ₂	k ₂₋₄
Samples not Incubated	0	1.53 × 10 ⁵	100.00		
	1	2.07 × 10 ³	11.70		
	2	1.98 × 10 ²	1.18	0.037	0.006
	3	1.12 × 10 ²	0.83		
	4	6.40 × 10 ¹	0.58		
Samples Incubated at 37°C for 24 Hours	0	1.13 × 10 ⁷	100.00		
	1	3.49 × 10 ⁵	27.80		
	2	6.30 × 10 ⁴	5.34	0.024	0.005
	3	3.54 × 10 ⁴	3.85		
	4	2.07 × 10 ⁴	2.86		

E. coli harvested in stationary growth phase, sprayed from distilled water suspension, at 30°C and 50% R. H. with samples collected in 0.2% buffered oxaloacetic acid.

The results of the experimental rate of death of airborne E. coli with reactivation are summarized in Table XI. The results indicated that all investigated metabolites, except succinic acid and malic acid, reactivated a significant number of bacterial cells which otherwise would have been considered dead because of their failure to grow in ordinary growth supporting medium.

It is possible, from the results obtained, to compare the percentage of recovery of living cells before and after incubation. Using Table X as an

FIGURE 28
 THE COMPARISON OF THE DEATH RATES OF AIRBORNE E. COLI
 WITH AND WITHOUT REACTIVATION BY EMPLOYING 0.2 PERCENT
 BUFFERED OXALOACETIC ACID SOLUTION
 (E. coli sprayed from distilled water suspension)

Logarithm of the Ratio of Living Cells at 0 Time and at the Time of Sampling

example, the percentage of recovery for the first hour, before incubation (without reactivation), was 11.7, and that after incubation (with reactivation) was 27.8. The ratio of recovery was $(27.8/11.7)$, or 2.36. The ratio of recovery for the second hour of storage was then $(5.34/1.17)$, or 4.52, for the third hour, $(3.85/0.83)$, or 4.64 and for the fourth hour, $(2.86/0.58)$, or 4.93. It can be seen that the ratio of recovery increases with an increase in percentage of death. In other words, with a greater number of apparently dead cells, the percentage of recovery was higher. This is somewhat expected since a greater probability of reactivation exists when more inactivated cells are subjected to reactivation. The same phenomenon was observed for all experiments using α -ketoglutaric acid and sodium citrate as reactivation agents.

TABLE XI

THE EFFECT OF REACTIVATION ON THE RATE OF DEATH OF AIRBORNE E. coli

Incubation Solution	Rate of Death, min. ⁻¹	
	k_2	k_{2-4}
Oxaloacetic acid	0.024	0.0050
Sodium citrate	0.022	0.0050
α -ketoglutaric acid	0.018	0.0040
Succinic acid	0.032	0.0054
Malic acid	0.034	0.0051
0.85% NaCl (control)	0.033	0.0060

E. coli harvested in stationary growth phase, sprayed from distilled water at 30°C and 50% R. H.

The results indicated that the desiccation of airborne E. coli had blocked the operation of the Krebs cycle in some cells to some degree, possibly by inactivating some enzymes involved in the synthesis of proteins. By supplying certain intermediate metabolites, the blocked reactions of the cycle were restored in some cells and the normal flow pattern of the cyclic operation was completely recovered.

J. The Stability of Airborne E. coli Aerosolized with Glutamic and Aspartic Acids

Since it was established that a portion of the death of airborne E. coli was the result of the interference of the synthesis of glutamic acid and of aspartic acid, the presence of either of these two acids in the aerosols would certainly help to stabilize the bacterial cells. In order to verify this fact, E. coli aerosols were sprayed from glutamic acid and aspartic acid solutions, both were prepared as 0.2 percent solutions with 0.1 M phosphate buffer, Na_2HPO_4 plus KH_2PO_4 . The death rates observed in these experiments definitely showed that airborne E. coli population could maintain life much longer than when sprayed from a distilled water suspension. Figure 29 compares the death rates affected by the presence of glutamic acid or aspartic acid. Death rates of E. coli aerosols sprayed from a culture medium suspension as well as from distilled water were also plotted for comparison. Evidently glutamic acid was the most effective in stabilizing airborne cells whereas aspartic acid and the culture medium had about the same effect.

Logarithm of the Ratio of Living Cells at 0 Time and at the Time of Sampling

FIGURE 29. THE COMPARISON OF THE DEATH RATES OF AIRBORNE E. COLI FROM DIFFERENT ORIGINS (IN LONG STORAGE PERIOD)

VII. DISCUSSION OF RESULTS

A. General Discussion

It was the purpose of this study to determine if one or more factors influence the death of airborne bacteria using a single species, E. coli. A number of investigators have observed that viability of airborne bacteria varied from genus to genus. In spite of this fact, it is felt that there should be some common factors affecting the viability of airborne cells. Webb's (10) data showed that S. albus, B. subtilis, S. marcescens and E. coli varied considerably in their death rates. All of these species indicated an extremely high rate of death during the first second of storage, while the death rate during the next 9 seconds was relatively slow. The same phenomenon was observed in the present study. This readily suggests one common mechanism which might be responsible for the rapid decay of all these bacteria, regardless of structural differences between the organisms.

The results also indicated that the highest mortality occurred at low R. H. values, with a decrease in rate of death with increasing R. H. Parallel to this phenomenon is the evaporation of a water droplet which takes place readily when it is exposed to an unsaturated air environment. Appendix A shows that a water droplet of 13 μ size at room temperature and R. H. evaporates to 0.9 μ diameter within four-tenths of a second. Consider an aerosol of about 13 μ size, composed of a distilled water layer surrounding a bacterial cell mass of 0.9 μ size, it would take therefore less than four-tenths of a second to evaporate the water layer completely and leave the bacteria cell suspended and exposed in the air. The evaporation of a water droplet at rest can be expressed by the following equation

$$\begin{aligned} \frac{dA}{dt} &= \frac{8\pi M}{R T d} (f \cdot p_T - p_\theta) r v \alpha \\ &= \text{Constant} \cdot \frac{v}{T} (f \cdot p_T - p_\theta) \end{aligned}$$

The half-second storage time, the time period after the bacterial cell has been entirely exposed to the air without the water layer appears to be critical to the viability of the airborne E. coli. The rate of death for this one-half second storage time has been shown in this study to be directly proportional to the decrease of R. H. when temperature is constant, Figures 12 and 14. The same relationship is found in the evaporation of a water droplet since at constant temperature, the equation becomes

$$\frac{dA}{dt} = \text{Constant. } (f \cdot p_T - p_\theta)$$

Furthermore, the rate of death of airborne cells during the first half-second storage period at constant R. H. was shown to be exponentially proportional to increasing temperature, Figures 13 and 15. It is important to note that the rate of evaporation of water droplet with varying temperature holds the same relationship, Figure 16.

Webb (10) observed a decrease in death rate with increased temperatures during the first second of aerosolization. This was, however, not observed in the present study. Webb explained his findings as being the result of a sudden chilling effect. It is obvious that the evaporation of water drops would result in a sudden lowering of the temperature at the surface of the drops. Therefore it is conceivable that some portion of the initial death of bacterial aerosol may be due to a large temperature drop. When the process of evaporation is carried out at a higher temperature the chilling effect is less.

Considering Equation 14 for temperature change,

$$\theta = \frac{L M D}{K R T} \frac{(f \cdot p_T - p_\theta)}{(D/rv\alpha + 1)} + T$$

the decrease in temperature, as calculated from this equation, would not lower

the droplet temperature down to 4°C at all the temperature and R. H. levels investigated in the present study, Table V. Hence, the sudden chilling effect on the bacterial aerosols cannot entirely account for their rapid initial death. The presence of bacterial cells in an aerosol could possibly reduce the evaporation coefficient α , so that the temperature decrease would be even smaller. Therefore, the calculation of the temperature decrease with α equal to 0.04 for the case of pure water gave higher values of temperature decrease (Table V).

The calculation of θ requires trial and error procedures which in themselves introduce some inaccuracy which is not significant for the present study. Also, the radius was taken as 13 μ in all calculations, though it was only true as an average condition. The following is a sample calculation of θ for a water droplet of 13 μ size in a 30°C and 50 percent R. H. environment.

$$\theta = \frac{579.5 \times 18 \times 0.255}{0.0000288 \times 62360 \times 303.8} \frac{(0.5 \times 33.321 - p_\theta)}{[0.255/(13/2) \times 10^{-4} \times 0.04 \times 14950 + 1]} + 303.8$$

$$= 4.87 \frac{(16.66 - p_\theta)}{1.655} + 303.8$$

By trial and error, it was found that at 21.7°C, p_θ was equal to 19.468,

$$\theta = 2.94 (16.86 - 19.468) + 303.8$$

$$= 295.5^\circ \text{ absolute temperature, or } 21.7^\circ\text{C}$$

Thus, the decrease in temperature was $303.8^\circ - 295.5^\circ = 8.3^\circ\text{C}$. The calculation of droplet temperature for other environmental conditions followed the same procedure. The results were tabulated in Table V. The lowest droplet temperature in the present study, as calculated, was 281.2° absolute degrees or 7.4°C, which was above 4°C. Therefore the lethal effect of a sudden chilling to 4°C was not applicable in interpreting the data collected in this study (48).

Since temperature and R. H. affect the viability of airborne bacteria simultaneously, it is necessary to study their combined effect. It was shown in Figure 17 that the rate of death of airborne E. coli was directly proportional to the rate of water evaporation. The rate of evaporation of protein-bonded water within cells is much different from the rate of water evaporation from a water droplet. The difference can be attributed to the fact that the diffusing water molecules were crossing boundaries of different thicknesses in completing the evaporation process. In the evaporation of water droplets, a water molecule diffuses from the spherical droplet into an imaginary boundary whereby the water molecule will have traveled a certain distance, Equation 7, before it collides with a gas molecule which completes the evaporation. Diffusion of a water molecule out of a bacterial cell is further complicated by the presence of the cellular membrane and the protein-water bond. The protein-water bond requires more energy in order for it to rupture before the water molecule is free to travel. The cell membrane adds to the effective thickness of the imaginary boundary through which the water molecule must pass before completing the course of evaporation. It is, therefore, evident that the rate of evaporation of water from within cells is much slower than that from water droplets alone.

From Figure 17, it appears that as the function of water evaporation becomes smaller, the spread of the individual points from the straight line becomes larger. This probably was due to the fact that at low temperature and high relative humidity, the evaporation of water was so slow that there was a significant lag period before the protein-bonded water could start diffusing out of the cell. As a result, the relationship between $k_{1/2}$ and the function of water evaporation would not hold. In the case of aerosols from saline water suspensions, this possible lag period did not exist because of the immediate

onset of dehydration of the cellular water. Therefore the straight line plot in Figure 18 was a perfect fit throughout all f values obtained in the study.

Glycerol in high concentration is "toxic" to bacteria, but at a concentration of 0.375 percent, it causes no harmful effect on bacteria. It is a trihydroxy alcohol highly hygroscopic in nature. When bacterial aerosols are sprayed using a glycerol suspension, water is not as readily evaporated as in aerosols sprayed from distilled water suspensions. Two different characteristics were encountered with airborne bacteria sprayed from glycerol suspension:

a. Owing to the hygroscopic nature of glycerol, the aerosols appeared to hold more moisture on the cells. Thus, a longer period of time was required to evaporate the water. In fact, it was found in the present study that E. coli suspended in 0.375 percent glycerol, as examined by a Carl Zeiss phase microscope, showed an average size of somewhere between $1\ \mu$ and $2\ \mu$, while the same culture suspended in distilled water showed an average size of less than $1\ \mu$. This indicated in glycerol suspension E. coli had a higher water content.

b. The glycerol as a trihydroxy alcohol would reduce the rate of water evaporation. It is a known fact that water, with added impurities, evaporates at a slower rate. As shown in Appendix A, the equation for calculating the time required for water evaporation indicates that anything which reduced the α value would result in a longer time for evaporation of the same droplet. It may be assumed, therefore, that glycerol reduced the α value. A reduction in the rate of water evaporation by fatty alcohols was reported by Eisner, Quince and Slack (44).

The results showed a significant decrease in the death rate, as anticipated. This suggests the theory that the rate of water evaporation governs the rate of death of bacterial aerosols.

The study of viability of airborne bacteria sprayed from a saline solution indicated a similar result compared to that obtained from aerosols of distilled water origin. The difference between the two was the slopes of the curves shown in Figure 30.

It was shown in the case of E. coli aerosols from saline solution that the rate of change in $k_{1/2}$ values was less than in the case of aerosols sprayed from distilled water. Another difference was that, at low temperature and high R. H., the $k_{1/2}$ values were higher in the case of bacterial aerosols from saline suspension, but the reverse was true at high temperatures and low R. H. as shown in Figure 30.

The mechanism of death of E. coli in both cases was identical. Regardless of the difference in slopes, the rate of water evaporation governed the rate of death. There were other factors besides temperature and R. H. which affected the evaporation of cellular water in the presence of sodium chloride. The presence of sodium chloride altered the rate of cellular water evaporation in the following ways:

a. After the water had been evaporated from the NaCl solution surrounding the E. coli aerosols, NaCl would form either a layer of crystals or, when the evaporation was not complete, a very high concentration layer of NaCl solution. In either case, the evaporation process was retarded because the thickness of the barrier through which a diffusing water molecule had to travel was increased. Sodium chloride also reduced water evaporation by lowering the vapor pressure in the solution (52).

b. "Dehydration" of protein molecules in the presence of high salt content accelerates water evaporation within cells. The higher the concentration, the more charged groups were formed and more polarized water particles were extracted from the cell proteins.

c. It has been mentioned that an osmotic pressure change can be tolerated by most bacteria to a certain extent. In spite of this fact, the

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osmotic effect was significant in the present study. As water evaporation proceeded toward completion outside the bacterial cells, the concentration of NaCl was so high that a very strong hypertonic solution existed. Consequently cellular water diffused out.

Effect (a) reduced the rate of water evaporation whereas effects (b) and (c) increased the rate of water evaporation. All these effects, including those of temperature and R. H., occurred simultaneously and interacted with each other. For example, a higher osmotic pressure accelerated the "dehydration" process; but as more water was diffusing out, the concentration of sodium chloride was prevented from increasing, and likewise the "dehydration" process. Therefore, there was an equilibrium condition when all these factors acted together, and the rate of water evaporation from cells under that specific condition was constant. When any one of the factors changed, a new equilibrium was formed which resulted in a new rate of death. This change of k values was at a slower rate in the case of saline suspensions than in the case of distilled water aerosols (Figure 30).

The fact that at low temperatures and high R. H. E. coli aerosols from saline water origin had a higher rate of death than those from distilled water origin can be explained as a result of a "dehydration" process. This process was much less affected by temperature and R. H. than by other factors. Although the protein-water evaporation effected by low temperatures and high R. H. was slow, especially with the presence of NaCl crystal outside the cell membrane, the "dehydration" process readily took place and this was lethal to the bacterial cells. This accounts for the rate of death being higher in the beginning. As the temperature increased and R. H. decreased, the limiting factor appeared to be the combined effect of temperature and R. H. on water evaporation. Figure 30 shows that at high temperatures and low R. H., the death rate was higher for E. coli aerosols of distilled water origin. This may be explained by the effect of NaCl crystals in increasing the boundary

layer thickness between the cells and the outer environment; as a result, water evaporation was retarded at a rate greater than the rate of increasing water evaporation by the effect of dehydration.

Figure 19 finally suggested that any influence on the rate of water evaporation had the same effect on the rate of death of airborne bacteria.

The phenomenon of an abrupt change in the death rate after one-half a second needs a little explanation. While a significant portion of the bacterial population died off in the first half-second of storage, it was surprising to find that many cells remained viable. It was believed that this phenomenon may be related to the site of protein-water bonds. Protein water can be linked to protein molecules through hydrogen bonds at various sites. Some of them are linked to the exterior of the protein molecule; others can be trapped within the interior of the native protein. Some are possibly vital and some not. Some protein molecules may not have water molecules in them. A significant number of the bacteria may have lost the protein water molecules which were vital, and died off during the first half-second of storage, while the remaining bacteria lost only non-vital bonded water, or had no bonded water to lose. Those cells which managed to survive may have had water molecules which were strongly bonded, or which were trapped within the interior of protein molecules. These water molecules would be very difficult to evaporate, not only because of the strong bond strength, but also because of their position inside the protein molecules which provided them with good protection against evaporation. The rate of evaporation would be, therefore, very slow. Consequently, the rate of death would also be very slow compared to that in the first half-second of storage. In fact, some of the bacteria could survive several hours probably because some protein-water was held inside indefinitely without evaporation.

According to this interpretation of the sudden change of death rate after approximately one-half second storage period, both the free water outside the bacterial cells and the bonded water linked to the exterior of the protein molecules were mostly evaporated within this period of time. However, the exact time when the vital bonded water molecules started diffusing out was not determined. Furthermore, no attempt has been made to determine the exact time when the relatively slow death rate began to occur. It is therefore not possible to compare the k values observed in this study with that found by other research workers.

In order to find out the exact time, samples would have to be obtained every one-tenth of a second or even a smaller fraction of a second. The design and precision of existing instruments make this type of study impossible at the present time.

It was postulated that the death rates found in the present study would follow the first order reaction. However, it was found not necessarily true for all cases. Many workers have also postulated partial order reactions and first to fifth order reactions (10). In the study of biological systems using death or certain inactivation as the end point for rate determination, the same reaction could involve different portions of a protein molecule at different rates. The breakage of one protein-water bond may cause death or inactivation whereas the breakage of one protein-water bond in another part of the same molecule may not be detrimental. Therefore, the lethal process could proceed along different paths to reach the same end point. The broken line in Figure 11 suggested this postulation.

The k_4 values were found to have an established relationship with the rate of water evaporation but not a linear one. This was somewhat expected because the bacterial aerosols at this stage were dehydrated to such an extent that at the temperature and R. H. ranges investigated, the relationship

between death rate and function of water evaporation did not hold, especially at low temperatures and high R. H. levels. The curves shown in Figure 23 are not straight lines.

To compare the death rate obtained in the short storage studies, Webb's data was used (10). However, no half-second sample was collected in his study. The whole first second storage period in the present study must be treated as an exponential function so that it was possible to compare results with Webb. At 25°C and 50 percent R. H., the k_1 for E. coli as calculated from Webb's data was 1.52. In the present study, k_1 at 20°C and 50 percent R. H. environmental conditions was calculated to be 1.14, and at 30°C and 50 percent R. H. environmental conditions, it was 1.35. The interpolated k_1 at 25°C and 50 percent R. H. was 1.24 which was slightly less than that found by Webb.

A study of the velocity of the aerosol flow in the system indicated no noticeable effect on the viability of bacterial aerosols within the range of velocities investigated. Kinzer and Gunn (39) have pointed out that for particles of very small size (Reynolds number between 0 and 7) the evaporation rate rapidly approached the characteristics of a drop at rest. They mentioned:

"Such a behavior is perhaps to be expected, since it is known that the motion of tiny drops is controlled not by the aerodynamic forces, but by the shear forces arising from the viscosity of the environment air. Because of the relatively large influence of viscosity, the shearing stresses close to the drop cause it to carry along entrained air that acts as though it were a part of the drop."

The initial average particle size of the aerosols used in the present study was 13 μ . This particle size fell in the range where its movement was entirely controlled by shearing stresses. From Kinzer's experimental curve presenting the relationship of K and $Re^{1/2}$ which exist in the wind factor equation as $f^1 = (1 + K \cdot Re^{1/2})$, this size of particle gives a K value of approximately zero, and the wind factor becomes unity. The result shown in Figure 20 verified

that the aerosols used in the present study had a rate of evaporation identical to those of water drops at rest.

The velocity of the flow in the short storage chamber was theoretically 14 inches per second as determined by a flow rate of 43 l/min and the cross sectional area of the chamber. The velocity of the flow in the chamber had been determined by a hot-wire anemometer to be very close to this value. Nevertheless slight variations in velocity in different positions of the cross section of the chamber were noticed. This indicated that the flow was not uniform throughout the whole cross section, and lower velocities could be expected near the wall of the chamber. When samples were being taken from the chamber, the flow pattern was altered. Hence, the half-second storage period of the gas stream calculated by the distance the aerosols traveled could be true only when the sampled aerosols traveled at a velocity of 14 inches per second. The velocity of flow near the wall where sampling points were situated was very likely less than 14 inches per second. So the actual storage time could have been longer than half a second for the first sample, and the $k_{1/2}$ values thus obtained may actually be higher values.

As storage time was extended in the storage system, most of the exteriorly linked water of the cell proteins was evaporated. From then on, the water evaporation from protein molecules did not affect the rate of death of cells in a linear relationship. The increasing of the temperature and lowering of the R. H. in the environment still provided the driving force for water evaporation from bacterial cells. However, because of the bond strength of those firmly held water molecules and their position in the cells, the rate of water evaporation did not increase proportionally to the increasing driving potential. Furthermore, the lack of growth supporting medium for synthesis complicated the situation in the long storage period. Figure 27 substantiates this conclusion.

The method of presenting the data from the long storage study by two death rates, k_2 for the first two hours and k_{2-4} for the subsequent two hours, must be explained. This method of analysis made it possible to compare the data with other research in this area of study. It should be noted that the interpretation of the data as two-component curves did not yield significant error. Webb (10) found that the rate of death for the first hour of storage was 0.032 min.^{-1} and that during the subsequent three hours storage it was 0.010 min.^{-1} in an environment of 25°C and 50 R. H. The present study indicated that at 30°C and 50 percent R. H., the rate of death for the first two hours of storage was 0.033 min.^{-1} and that for the next two hours was 0.006 min.^{-1} .

In order to carry on the necessary metabolic function, bacterial cells need a growth supporting medium. This is no exception for cells in an airborne state. For a storage time as long as a few hours, bacterial cells will multiply in the presence of utilizable metabolites (a standard generation time for E. coli in suitable growth medium in solution form is 20 to 30 minutes). On the other hand, bacterial cells will die off within this storage period without the presence of utilizable metabolites. The number of living bacterial cells recovered at the end of the storage period would then be very different in these two cases. The rate of metabolism of airborne cells at room temperature and R. H. is perhaps quite different to that under the optimum growth condition, 37°C in a solution of growth supporting medium. It can be assumed that in an airborne state, most of the water content in an aerosol is evaporated, and the moisture which is available to the synthesis process is very limited. Even in the presence of growth supporting medium, the metabolism would be greatly retarded as a consequence of the limited amount of water available for the diffusing of metabolites into the cells and waste products out from the cells. Table IX verified that in the presence of growth supporting

medium, E. coli aerosols were stabilized because the rate of death was less than that when a growth supporting medium was absent.

There exists a possibility that the stabilization caused by the presence of growth supporting medium interfered with water evaporation. However, the culture medium had about the same salt concentration and osmotic pressure of a 0.85 percent sodium chloride solution. It was found that with this salt concentration in the bacterial suspension and at 30°C and 50 percent R. H., E. coli aerosols actually had a higher rate of death than that of E. coli aerosols from distilled water origin. It was found in the short storage study that at 30°C and 50 percent R. H., the water evaporation function, f , was 144.50 (Table V). Both Figure 23 and Figure 30 showed that E. coli aerosols died more rapidly when they were sprayed from 0.85 percent saline solution than in the case of aerosols produced from distilled water. The lower death rate in the presence of growth supporting medium was then the result of the ability of E. coli aerosols to utilize the metabolites for multiplication.

Since it appears that airborne cells at room temperature and R. H. utilize growth supporting medium for growth, the effect of growth phases of bacteria on their viability must be considered. Bacterial cells in different phases of growth possess different rates of metabolism. When a definite number of cells are sprayed into the chamber, active cells utilize the metabolites readily and multiply accordingly. Bacterial cells which are less active do not utilize the metabolites as readily and rapidly as the active cells do, and consequently less new cells will be synthesized for the same storage period. Since death rates are determined by comparing the number of living cells before and after the storage period, the death rate of active cells should be smaller than that of less active cells because more living cells can be recovered in the former than in the latter case. Table IX showed the difference of death rates, though it was not very significant because this was due to the limited

amount of moisture present. The limited amount of moisture in the bacterial cells retarded the synthesis process so that the difference of the rate of metabolism was largely counterbalanced.

Glucose itself as an exogenous energy source can support bacterial growth in solution. No definite conclusion can be reached from the results of the present study to substantiate this same phenomenon for airborne bacteria. Although the death rate of cells sprayed with 0.2 percent glucose was observed to be slightly less, the difference was so small that it could have been the result of a decreased rate of water evaporation from aerosols because of the presence of glucose in the droplets.

It is widely accepted that the Krebs cycle is the major mechanism for the synthesis of aspartic acid, glutamic acid and the group of amino acids derived from them. As these amino acids contain more than 50 percent of the protein carbon of E. coli cells (12), it is apparent that the Krebs cycle is the major mechanism for producing the necessary compounds for protein synthesis. The operation of the Krebs cycle is also observed in other organisms, various kinds of yeast, mold and algae. In the present study the flow pattern of the Krebs cycle was used to demonstrate the ability of some metabolites to reactivate airborne bacterial cells. This demonstrated that inactivation of certain protein molecules due to the lack of these metabolites or to desiccation in airborne bacteria was responsible for the death of cells, and that the responsible metabolites could restore the activity of bacterial cells. Although the Krebs cycle is not the mechanism synthesizing the necessary amino acids for all the proteins in the cell, it is felt that the same phenomenon of inactivation and reactivation of protein molecules affecting the activity of cells occurs in protein molecules other than those derived from the Krebs cyclic operation. This means that other amino acid families such as serine, histidine and aromatic amino acids or their precursors could be inactivated and reactivated,

and the activity of the bacterial cells is affected accordingly.

A Krebs cycle is shown in the following to facilitate discussion regarding the mechanism of the reactivation process:

The metabolites investigated were:

- Oxaloacetic acid
- Citric acid (Sodium citrate)
- α -ketoglutaric acid
- Succinic acid
- Malic acid

Since oxaloacetic acid and α -ketoglutaric acid are immediate precursors of aspartic acid and glutamic acid respectively, they are most important insofar as their positions in the operation of the Krebs cycle are concerned. Results showed that both oxaloacetic acid and α -ketoglutaric acid, particularly oxaloacetic acid, restored the activity of some inactivated bacterial cells if a proper incubation period of approximately 24 hours at a temperature of 37°C was provided. This observation suggested two possibilities of what may have happened to the airborne bacteria in the study:

1. The synthesis of aspartic acid and of glutamic acid in the cells was blocked so as to cause inactivation of the cells. Supplemented with their immediate precursors which would induce enzyme formation for the synthesis, these two amino acid compounds could be synthesized and the activities of cells were partially reestablished.

2. The formation of oxaloacetic acid and of α -ketoglutaric acid was blocked. When they are supplied as exogenous metabolites, the operation of the Krebs cycle was restored during the incubation time.

The concept of reactivation of "killed" bacteria here suggests that some basic functional activities of the cellular processes were left essentially intact during the lethal process of desiccation. The cells may regain activity if they are brought to function specifically to reestablish cellular division. Two modes of action of the metabolites investigated in this study can be suggested in justifying the reactivation of "killed" bacteria. The first restores the inactivated enzyme systems necessary for metabolism. Knox et al. (50), working with E. coli, demonstrated that chlorine in bactericidal concentrations reduced the activity of various sulfahydril enzymes and other enzymes which were sensitive to oxidation. Oliver (51) found that the activity of the decarboxylating enzymes of E. coli ceased above 80°C but resumed their activity on return to lower temperatures. He also observed that

E. coli in suspensions "killed" by heat and chemicals were still sterile after 7 days' incubation in culture medium but possessed almost normal decarboxylase activity. This indicated that loss of viability did not represent the loss of all enzyme activity. All those experiments reported by previous workers, as well as the experiments in the present study, support a speculation that if a route of entry into the respiratory cycles could be found with a proper metabolite, a partial restoration of cyclic process could take place. Such a phenomenon could be explained as a stepwise operation in a chain process where a metabolite induces enzyme formation, and subsequent enzymatic conversion of metabolite would provide a new metabolite for other enzyme formation. In such a manner, a complete cycle could become operative. The second or alternate mode of action of the metabolites in reactivation could be interpreted as providing a supply of more readily available energy. The supplemented tricarboxylic acids which are immediately utilizable compounds would serve as energy sources.

It was also shown that citrate was able to restore partially the inactivated airborne bacteria whereas succinic acid and malic acid did not show any definite reactivation ability. Table XI showed that α -ketoglutaric acid was most effective in reactivation, sodium citrate being less effective and oxaloacetic acid the least effective among the three. The result can be interpreted with respect to the positions of these compounds in the Krebs cycle operation. Since oxaloacetic acid and α -ketoglutaric acid are immediate precursors of aspartic acid and glutamic acid respectively, which are the primary compounds for protein synthesis in E. coli, their ability to reactivate "killed" cells is readily visible. Regardless of whether protein molecules or enzyme systems are inactivated, oxaloacetic acid and α -ketoglutaric acid are in a closer position with the amino acids and protein products, and the presence of these two compounds certainly gives a higher efficiency of reactivation.

Between aspartic acid and glutamic acid, the former is a much better amino acid donor (12). It seems that internally synthesized aspartic acid acts as an amino acid donor in many transamination reactions, even in the absence of exogenous aspartic acid. On the other hand, glutamic acid is a relatively poor amino acid donor, and needs more exogenous supply to carry on the metabolism. The supplement of α -ketoglutaric acid replenishes the glutamic acid and very likely induces enzyme formation in order to clear the pathways for metabolism. It was also shown that a large fraction of exogenous glutamic acid can be converted into aspartic acid through the Krebs cycle operation (12). Consequently, supplemented α -ketoglutaric alone could restore partially the cyclic flow and therefore reestablish activities of the "killed" bacteria. Though the supply of exogenous oxaloacetic acid alone may induce the formation of aspartic acid, it has to be incorporated with an exogenous acetate supply in order to carry out further steps in the Krebs cycle. Without the acetate supply, the synthesis of glutamic acid is significantly retarded. Therefore the efficiency of the reactivation process carried out by oxaloacetic acid alone cannot be expected to be as great as that by α -ketoglutaric acid. When exogenous citrate is supplied, the Krebs cycle is carried on with the formation of glutamic acid which in turn produces aspartic acid. As a result, it gives a better efficiency of reactivation than oxaloacetic acid. Succinic acid and malic acid were also supplied in the present study as exogenous sources for the purpose of demonstrating reactivation. Their positions in the Krebs cycle, however, are such that they do not lead to the synthesis of aspartic acid and glutamic acid as readily and directly as oxaloacetic acid. This makes them extremely ineffective to restore the cyclic operation if glutamic acid needs to be supplied.

From the discussion above, it is felt that the inactivation of airborne cells does not occur specifically in some particular protein molecule or enzyme systems. Very likely most of the proteins and enzymes involved in the

bacterial synthesis are partially damaged because of broken protein-water bonds and lack of readily utilizable metabolites. Therefore, each investigated metabolite had more or less some effect in reactivation. The degree of activation appears to depend upon how readily the metabolite can be utilized to synthesize aspartic acid as well as glutamic acid. The glutamic acid synthesis is particularly important because internal synthesis of glutamic acid is not as good an amino acid donor as aspartic acid. The exogenous aspartic acid supply may be deficient, but the same acid compound synthesized internally can carry on the cycle operation though the rate of the flow in the operation is much less.

Since aspartic acid and glutamic acid were of primary concern in the reactivation of bacterial cells, the last experiment was designed to see how their presence in the bacterial aerosols could affect the viability of air-borne E. coli. It was observed that both aspartic acid and glutamic acid stabilized the airborne E. coli. Glutamic acid was the most effective while aspartic acid was about as effective as the growth supporting medium. The stabilization phenomenon may be attributed either to the presence of readily available energy for growth in the storage period, or to the effect of reactivation. Since in this experiment no incubation was required for the samples before pouring agar plates, both acid compounds were probably more important in supplying an available energy source than in supplying metabolites to induce enzyme formation for reactivation.

Some practical aspects of the phenomenon of stabilization and reactivation of airborne bacteria certainly deserve attention. If the sterility of an air environment is tested by culture plate or culture tube techniques, the sterility is only arbitrary because the present study reveals that suitable metabolites could recover inactivated bacterial cells when incubated for a certain period. Air hygiene such as the disinfection of air by ultraviolet

light or by chemical agents would not ensure the safety of the "cleaned" air. A large amount of "killed" pathogenic bacteria might enter the body of a host and, through proper incubation in the presence of some suitable metabolites, be reactivated and resume their multiplication process. The sterilizing of an air environment perhaps should be raised to such a standard that metabolic recovery and metabolic stabilization would fail in order to secure complete sterility.

B. Future Works

From the author's experience in the present study, it is felt that the particle size associated with aerosols containing bacteria affect the viability of cells. More bacterial cells in one aerosol for example would protect each other from rapid cellular water evaporation. It is suggested that works should be done in the future concerning this aspect. Also, it seems important that studies of the bacterial stabilization in the presence of amino acids should be carried out more intensively in the future. A study covering a variety of amino acids should be done in order to determine a general significance. Another aspect worth studying is the reactivation of airborne bacteria after sterilization by different disinfectants, such as sodium hypochlorite, resorcinol, propylene glycol, or by physical treatment such as exposure to ultraviolet light. Standard concentrations of these disinfectants should be investigated in different environments so that no reactivation with metabolites can be demonstrated. Finally, different kinds of bacteria should be studied, including the most frequently found airborne pathogenic bacteria, so as to fulfill the practical purpose of controlling the viability of airborne bacteria.

VIII. CONCLUSIONS

A. Factors Affecting the Viability of Airborne Bacteria During Short Storage

1. The mechanism of instantaneous killing of airborne bacteria is related to the evaporation of cellular water. This finding and the results of other research workers suggest that this mechanism can be applied to all kinds of airborne bacteria.

2. Relative humidity in the environment determines the equilibrium water content in airborne bacteria. It also determines the rate of transfer of water vapor between cell surface and the environment. The rate of death of some airborne bacteria is directly proportional to the dryness of the environmental air. A linear relationship exists between the rate of death and the dryness of the air.

3. Temperature governs the rate of change of water vapor as well as the rate of exchange of heat between the cell surface and the environment. Therefore it affects the viability of airborne bacteria. The rate of death increases exponentially with an increase in temperature.

4. The rate of death of airborne bacteria is directly proportional to the rate of cellular water evaporation in an instantaneous lethal process.

5. Foreign material associated with bacterial aerosols either increases or decreases the rate of death of airborne bacteria by changing the rate of water evaporation from within the cells.

B. Factors Affecting the Viability of Airborne Bacteria During Long Storage

1. The rate of death also increases with an increased rate of water evaporation, but a linear relationship does not hold. Other factors affect the viability simultaneously.

2. The viability of airborne bacteria is affected by the presence of a growth supporting medium. At 30°C and 50 percent R. H., airborne bacteria are capable of synthesizing food material and therefore more living cells can be recovered. Consequently, the rate of death is less.

3. When a growth supporting medium is present, the growth phase of bacteria also influences the viability of airborne bacteria. Active cells, in the accelerating growth phase, appear to have a slower death rate because more new cells are synthesized to offset the number of dying cells.

4. In the presence of amino acids which can be readily utilized as intermediate metabolites by the airborne cells, the viability is greatly increased.

4. Reactivation of Airborne Bacteria

Inactivated airborne bacteria can be recovered with proper incubation in the presence of intermediate metabolites. The degree of disinfection of an air environment should be raised to such an extent that metabolic recovery could fail.

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APPENDIX A

THE CALCULATION OF THE TIME PERIOD REQUIRED TO EVAPORATE
A WATER DROPLET FROM 13 μ DIAMETER DOWN TO 0.9 μ^* DIAMETER

By using equation (15)

$$\frac{dA}{dt} = \frac{8\pi M}{RTd} r v \alpha (f \cdot p_T - p_\theta)$$

where A = surface area of water droplet

α = evaporation coefficient, 0.04 for pure distilled water

d = density of water vapor, =1

f = relative humidity expressed as a fraction

M = molecular weight of water, 18

p_T = water vapor pressure at ambient absolute temperature

p_θ = water vapor pressure at equilibrium temperature

r = radius of water droplet

R = universal gas constant, 62,360 cc mm/mol. deg.

t = time in seconds

T = ambient absolute temperature

$v = (RT/2\pi M)^{1/2}$, a constant at constant temperature.

Since $A = 4\pi r^2$, substitute into the previous equation,

$$\frac{d(4\pi r^2)}{dt} = \frac{8\pi M}{RTd} r v \alpha (f \cdot p_T - p_\theta)$$

$$8\pi r \frac{dr}{dt} = \frac{8\pi M}{RTd} r v \alpha (f \cdot p_T - p_\theta)$$

* 0.9 μ is the equivalent size of the bacterial cell mass in an average size aerosol of 13 μ dia. (see Table I).

Rearrange and integrate,

$$\int_0^t dt = \frac{RTd}{Mv\alpha (f \cdot p_T - p_\theta)} \int_{r_0}^{r_t} dr$$

or

$$t = - \frac{RTd}{Mv\alpha (f \cdot p_T - p)} \frac{r_0 - r_t}{p}$$

where droplet temperature θ and the corresponding vapor pressure p_θ can be calculated by using equation (14),

$$\theta = \frac{LMD}{KRT} \frac{(f \cdot p_T - p_\theta)}{(D/rv\alpha + 1)} + T$$

At any instant, for example at 30°C and 50 percent R. H.

$$\theta = 295.5^\circ \text{ absolute temperature,}$$

and

$$p_\theta = 19.468 \text{ mm Hg. (see page 77)}$$

$$t = - \frac{62,360 \times (303.8) \times 1}{18 \times 14,950 \times 0.04} \frac{(13/2 - 0.9/2) \times 10^{-4}}{[1/2 \times (33.321) - 19.468]}$$

$$= 0.38 \text{ second}$$

APPENDIX B

RELATIVE HUMIDITY DETERMINED BY WET BULB AND DRY BULB TEMPERATURE

(Figures in the table are the wet bulb temperature readings that go with the corresponding dry bulb temperatures to give the proper relative humidities)

Dry Bulb Temp.	R. H.						
	20%	30%	40%	50%	60%	70%	80%
20°C	9.3	11	12.5	13.8	15.2	16.3	17.8
68°F	48.8	51.8	54.3	56.9	59.3	61.6	64
30°C	16.2	18.0	20.0	22.1	23.9	25.5	27.1
86°F	61.2	64.3	68.1	71.8	74.9	78.0	80.9
40°C	22.1	25.1	27.9	30.3	32.5	34.8	37.0
104°F	71.7	77.2	82.1	86.6	90.7	94.4	98.0
50°C	29.0	34.0	36.0	39.0	42.0	45.0	47.0
122°F	85.0	94.0	97.0	103	108	113	116
60°C	36.0	42.0	46.0	49.0	52.0	54.0	57.0
140°F	97.0	108	115	120	126	130	134

APPENDIX C2

RELATIVE COUNTING RATE CURVE

VITA

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