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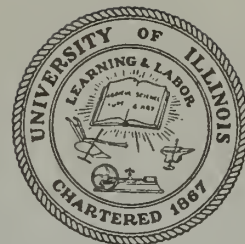
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# STUDIES ON COAGULATION EMPLOYING AMMONIUM CHLORIDE AND OTHER AEROSOLS

PROGRESS REPORT: June 1, 1962

Through August 31, 1963

By

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ICHIYA HAYAKAWA, DIRECTOR

DEPARTMENT OF CIVIL ENGINEERING  
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URBANA, ILLINOIS

September 10, 1963





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## SUMMARY

The principle findings of Part 1 "THE EFFECTS OF HUMIDITY ON THE COAGULATION OF AMMONIUM CHLORIDE AEROSOLS" are as follows:

(1) The weight concentration of air-borne particles with stirring was checked, using Nessler's reaction, revealing a decrease at the rate of a second order reaction, thus showing coagulation to be the main factor in the decrease of weight concentration.

(2) Water vapor was shown to decrease the stability of an aerosol of a one percent ammonium chloride solution. With a relative humidity of 50% or less, water vapor affected the coagulation only slightly more. When the relative humidity was 55% or more, water vapor markedly decreased the stability of an aerosol of a one percent ammonium chloride solution.

(3) The effect of foreign vapor on the coagulation of an aerosol was evident not only in the case of solid particles, but also with aerosols of liquid particles.

In addition, Part 2 "SHORT STORAGE STUDY ON THE VIABILITY OF AIRBORNE BACTERIA" has been studied. Data collected to date indicate a very high rate of death during the first 0.5 second of storage. This may be the result of a rapid evaporation of the bound water within the bacterial cells. With a longer storage period, the death rate appears to decrease. Different factors affect the viability of airborne bacteria. Temperature, relative humidity, source of aerosol and growth phase of the bacteria are considered important and preliminary investigations with various combinations of these factors have been conducted.



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Part I. THE EFFECTS OF HUMIDITY ON THE COAGULATION RATE  
OF AMMONIUM CHLORIDE AEROSOLS

I. Hayakawa  
T. Nirei

I. INTRODUCTION

In these studies the influence of humidity on the coagulation process has been investigated. The values obtained experimentally by others for the coagulation of various aerosols were generally in agreement with the value calculated from Smoluchowski's theory on the assumption that the collision efficiency between the particles is 100% (1, 2, 3). Though some workers have reported a stabilizing effect of a particular vapor on some aerosols, others have found either no effect or a decrease of the stability, for the same system.

In an earlier paper <sup>(4)</sup>, the effects of various foreign vapors (non-polar compounds, neutral polar compounds, and acidic polar compounds) on the coagulation rate of ammonium chloride aerosols were studied. This work provided the background for the present studies involving the effects of humidity on the coagulation rate of ammonium chloride aerosols. A technique termed the light scatter decay method, which involves a simplified analysis of the change in light intensity of a Tyndall beam as an aerosol settles under turbulent conditions, was used in both series of experiments.

These studies on coagulation employing ammonium chloride aerosols clearly showed that non-polar compounds and neutral polar compounds increased the stability of an aerosol of ammonium chloride, whereas acidic polar compounds decreased the stability of the same aerosol. Smoluchowski's theory has been shown not to be universally applicable.





On the basis of these studies it is felt that additional studies of the effects of humidity on the coagulation rate of ammonium chloride aerosols would be of great value from the practical as well as the theoretical standpoint.

The suggestion has been made that a thin layer of vapor or even liquid absorbed on aerosol particles might alter their surface. Smironov and Solntseva <sup>(5)</sup> reported the effectiveness of water vapor as well as butyric acid as aggregants for an ammonium chloride aerosol, but Samokvalov and Kozhukhova <sup>(6)</sup> stated that either water or octyl alcohol in low concentrations acted as a stabilizer, rather than as an aggregant, for ammonium chloride. However, Dalla Valle, Orr, and Hinkle <sup>(7)</sup> reported that water vapor produced the greatest aggregation of such aerosols.

The purpose of the studies herein reported were to investigate whether the coagulation of aerosols was a second order reaction or not, and some of the aspects of aggregation with an emphasis on examining the effects of humidity in the aerosol system.



## II FORMATION OF AEROSOLS

Consideration is given to two methods by which particulate clouds are formed:

- (a) condensation method, in which clusters of molecules come together to build up particles of colloidal dimension;
- (b) desparation methods, in which a substance, initially in bulk or in a state of relatively coarse subdivision, is further split up into fine particles.

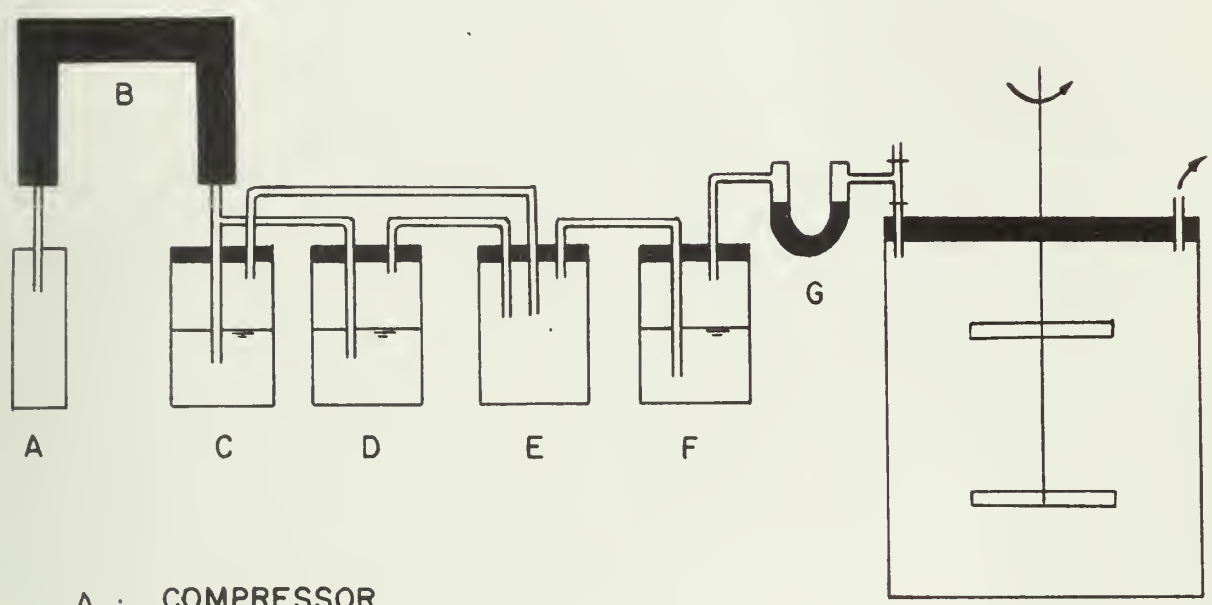
(a) Chemical Reaction (Condensation Method)

In the laboratory, the generation of aerosols by chemical interaction in the gas phase is very convenient. Certain gases or vapors react chemically with one another to form products which have a very low vapor pressure at ordinary temperatures, e.g.,  $\text{NH}_4\text{Cl}$  fume from  $\text{HCl}$  and  $\text{NH}_3$  (Figure 1);  $\text{H}_2\text{SO}_4$  mist from  $\text{SO}_3$  and water vapor. Since the gases are molecularly dispersed, the new particles must first be in a molecularly dispersed condition. The newly formed molecules aggregate and condense to form very fine liquid or solid primary particles. The formation of a mist or fume by the chemical interaction of two or more gases, therefore, is essentially a condensation process.

The general arrangement of the apparatus for the chemical generation of ammonium chloride aerosol is shown in Figure 1. Dry air filtered through glass wool, is bubbled through concentrated ammonium hydroxide (D) and concentrated hydrogen chloride (C) by the use of an air compressor (A). Gaseous ammonia and hydrogen chloride are mixed in the reaction bottle (E) where the ammonium chloride aerosol is produced. The mixture of gas and aerosol then passes through the water bottles (F), which act as impingers, and enters the smoke box (S).



### SCHEMATIC DIAGRAM OF AEROSOL GENERATOR (CHEMICAL REACTION)



- A : COMPRESSOR
- B : FILTER
- C : HCl (HYDROGEN CHLORIDE)
- D :  $\text{NH}_4\text{OH}$  (AMMONIUM HYDROXIDE)
- E : REACTION BOTTLE
- F : WATER BOTTLE
- G : SODA-LIME
- S : SMOKE BOX AND STIRRER



The water bottles are used for the purpose of "obligatory liquid filtration" (8). A simple experiment will aid in clarifying the point. Suppose that an aerosol is dispersed by an ordinary atomizer from a dye solution (eosin, fuchsin, toluidine blue, etc.). This aerosol is passed through an impinging series of Erlenmeyer bottles containing distilled water, which was the solvent used to prepare the mother solution. Six to eight of these washing bottles are placed in series and the successive coloration phenomena of the water in the different bottles can be easily observed. The first is intensely colored, the second less, the third still less, and so on up to the sixth, seventh, or perhaps the eighth (according to the dispersion efficiency of the atomizer, the air flow, etc.). There always comes a time when the water in a given one no longer colors. Now if at this moment the aerosol, issuing from the bottle whose water is not colored, is collected with an electric or thermal precipitator, particles whose size is extremely small and of great uniformity are regularly found in the air sample. On this principle three impinging water bottles are used to take out extremely large particles of ammonium chloride aerosols and excess ammonia (or hydrogen chloride).

In this case, the volume of air from the air compressor flows at the rate of 1.0 l/min. and the concentration of the ammonium chloride aerosol, which enters the smoke box (S) in one minute, is about 11 mg/l. This was checked with Nessler's reaction.

Reproduction of aerosol size and concentration is very important for the experiment and physical factors were kept as uniform as possible. The temperature of compressed air, hydrogen chloride, ammonium hydroxide, a reaction bottle and water in water bottles were kept constant and uniform. The pressure of the compressed air must likewise remain constant. A new sample of an ammonium chloride aerosol was made for every experiment.





(b) Air Atomizer (Dispersion Method)

Since 1920, it has been shown by a number of research workers: that the size of the micellae formed from ordinary atomizers varies with the salt concentration of the generating solution, being greater the more concentrated the solution, regardless of the nature of the dissolved material <sup>(9)</sup>. With the atomizer used by Stalport <sup>(10)</sup>, for example, the mean diameters of the particles (under an optical microscope) were:  $1.6\mu$  for 10% solution,  $1.06\mu$  for 1% solution,  $0.71\mu$  for 0.5% solution and  $0.37\mu$  for 0.1% solution. With an air-liquid jet Lauterback and his co-workers <sup>(11)</sup>, examining the aerosols it produced, found that when the jet was working above the solution surface, the mass median diameters of sodium chloride crystals were  $0.9\mu$  for 10% solution,  $0.4\mu$  for 1% and  $0.2\mu$  for 0.1%. The median count diameters were respectively  $0.08\mu$  for 10%,  $0.04\mu$  for 1% and  $0.03\mu$  for 0.1% sodium chloride solutions. When the jet was submerged, that is, when there was some water scrubbing of the large particles, the mass median diameters, for the same solutions, were respectively  $0.7\mu$ ,  $0.3\mu$  and  $0.2\mu$ .

In these experiments an aerosol of an ammonium chloride solution was made by the following method:

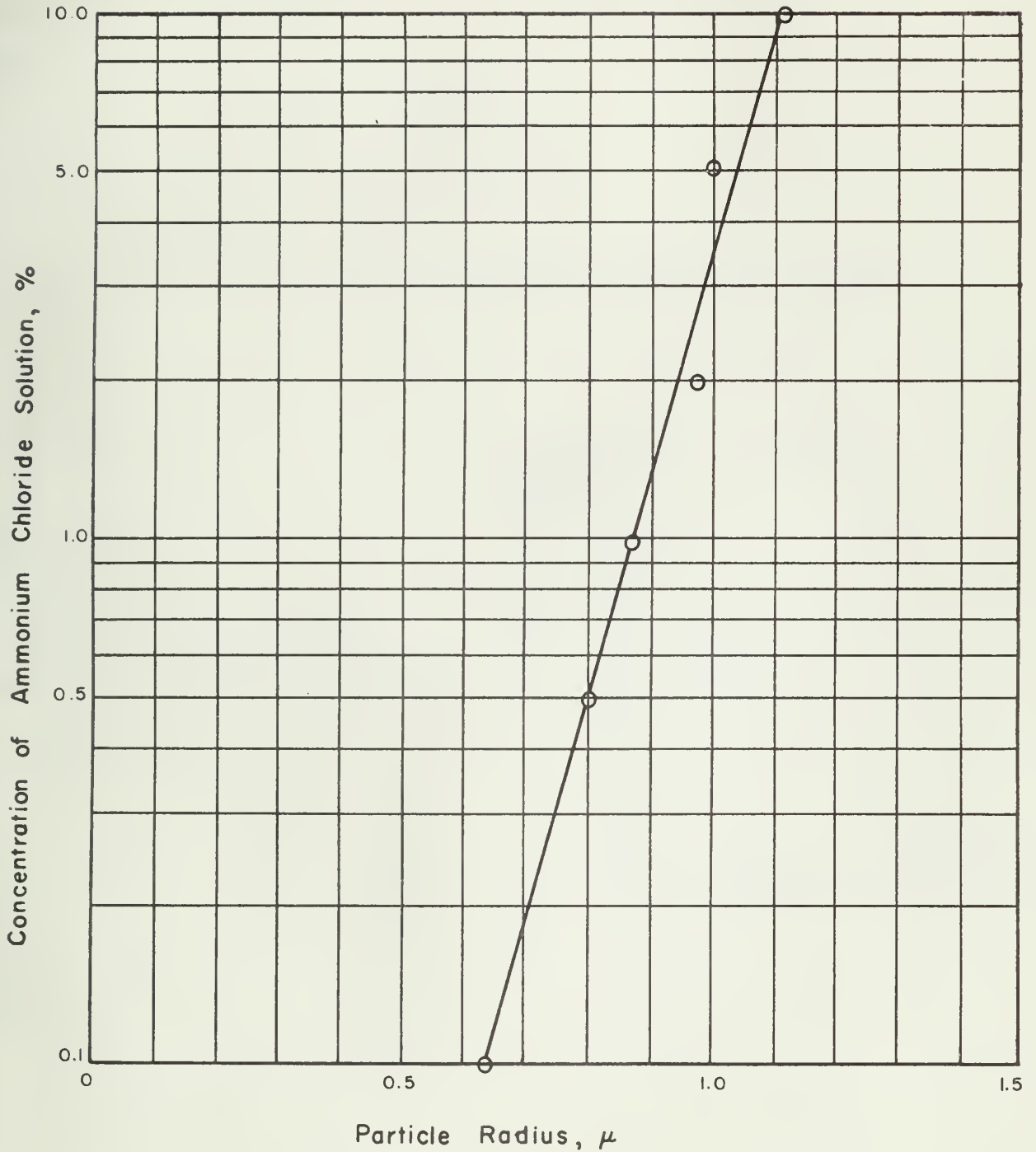
- (a) A one percent solution (weight) of ammonium chloride was prepared; and
- (b) Aerosols were created by a Wells-type atomizer <sup>(12)</sup> under 5 p.s.i.g. air pressure and 5 l/min. air flow.

The relationship between the concentration of ammonium chloride solution and the size of aerosol was checked by the light scatter decay method described in my earlier paper <sup>(13)</sup>. The results were shown in Figure 2.



Figure 2

RELATIONSHIP BETWEEN PARTICLE SIZES  
AND CONCENTRATION OF AMMONIUM CHLORIDE SOLUTION  
USING A WELLS-TYPE ATOMIZER





## III. EXPERIMENT

## (a) Calculation of Concentration During Inflow

The number of particles per cubic centimeter for the experiment must be less than  $10^6$  particles/cm<sup>3</sup> (14). The following calculation was applied for the experiments.

## Nomenclature

V: Volume of cloud chamber, 125,000 cm<sup>3</sup>

K: Concentration of the flowing aerosol, g/cm<sup>3</sup>

Q: Volume rate of flow, cm<sup>3</sup>/min.

C: Concentration of the aerosol in the cloud chamber at time t, g/cm<sup>3</sup>

t: Time, min.

$$V \frac{dc}{dt} = Q K - Q C$$

$$\frac{dc}{dt} = \frac{Q}{V} (K - C)$$

$$\frac{dc}{(K-C)} = \frac{Q}{V} dt$$

$$-\ln (K - C) = \frac{Q}{V} t + \text{constant}$$

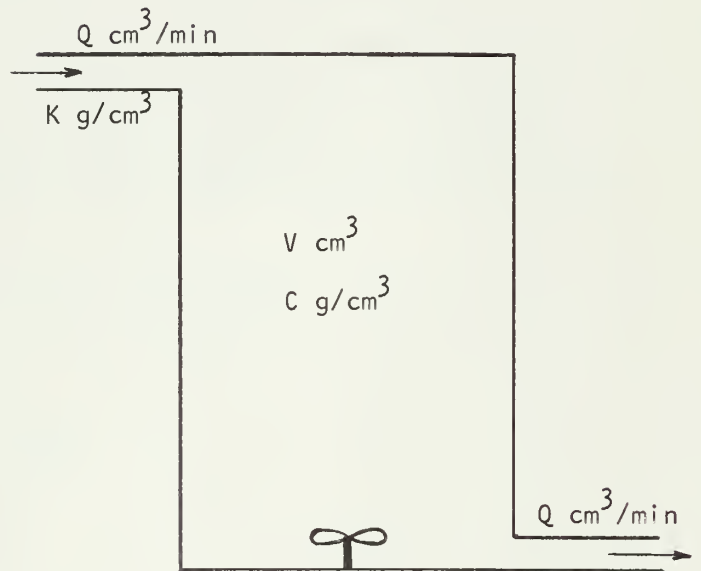
when  $t = 0$ ,  $C = 0$ , constant =  $-\ln K$

$$\ln \frac{K}{(K - C)} = \frac{Q}{V} t$$

$$\frac{K}{K - C} = e^{\frac{Q}{V} t}$$

$$\frac{K - C}{K} = e^{-\frac{Q}{V} t}$$

$$\frac{C}{K} = 1 - e^{-\frac{Q}{V} t}$$





$$= 1 - 1 + \frac{Q}{V} t - \frac{\left(\frac{Q}{V} t\right)^2}{2} + \dots$$

$$\frac{C}{K} = \frac{Q}{V} t$$

$$C = \frac{Q}{V} K t$$

In these experiments the Wells-type atomizer atomized approximately one cubic centimeter of fluid of a one percent ammonium chloride solution in ten minutes. Therefore,

$$\begin{aligned} C &= \frac{Q}{V} K t \\ &= \frac{1}{125,000} \text{ g/cm}^3 \end{aligned}$$

if we assume the concentration of particles was a fifty percent solution, because water evaporates from particles.

$$C' = \frac{1}{125,000} \times \frac{1}{50} = \frac{1}{6,250,000} = 1.6 \times 10^{-7} \text{ g/cm}^3$$

The number of particles per cubic centimeter for the experiments of the light scatter decay method may be calculated knowing that one gram of a one percent ammonium chloride solution was dispersed in a 125 liter cloud chamber. Therefore,

$$\rho = \frac{50}{100} \times 1.53 + \frac{50}{100} \times 1.00 = 1.26 \text{ g/cm}^3$$

$$\begin{aligned} V &= \frac{4}{3} \pi r^3 \\ &= \frac{4}{3} \times 3.14 \times (0.85 \times 10^{-4})^3 \\ &= 2.57 \times 10^{-12} \text{ cm}^3 \end{aligned}$$

where  $\rho$  = density of particle

$V$  = volume of a particle

$r$  = radius of a particle =  $0.85\mu$  (assuming)

$$W = V\rho = 2.57 \times 10^{-12} \times 1.26 = 3.24 \times 10^{-12} \text{ g}$$

$$n = \frac{C'}{W} = \frac{1.6 \times 10^{-7}}{3.24 \times 10^{-12}} = 0.49 \times 10^5 \text{ particles/cm}^3$$



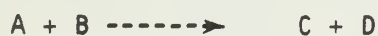


## (b) Nessler's Reaction Method (Weight Concentration)

For observation of particles by the Nessler's reaction method, aerosols of ammonium chloride were prepared with a chemical reaction type aerosol generator (Figure 1), and a rather polydispersed aerosol was produced.

When the supply of aerosol was stopped, the weight concentration =  $C_0$ . After  $t$  minutes the weight concentration =  $C_t$ . Figure 3 (a) prepared from experimental data of Table 1 gives non-linear curves for the relationship between  $\frac{C_0 - C_t}{C_0}$  and time. Figure 3 (b), on the other hand, shows a straight line relationship between  $\frac{C_0 - C_t}{C_0 C_t}$  and time. This means that the reaction is of the second order, i.e.,  $\frac{C_0 - C_t}{C_0 C_t} = K_2 t$  as shown below.

Because the general second order equation is (15):



$$\frac{dx}{dt} = K_2 (a - x) (b - x)$$

$$\frac{1}{a - b} \ln \frac{b(a - x)}{a(b - x)} = K_2 t$$

A special case of the general second order equation arises when the initial concentrations of both reactants are the same,  $a = b$ . This concentration can be purposely arranged in any case, but it will be necessarily true whenever only one reactant is involved in a second order reaction. When  $a = b$ ,  $K_2 t$  is indeterminate. It is best to return to the differential equation, which

becomes  $\frac{dx}{dt} = K_2 (a - x)^2$  or  $\frac{dx}{(a - x)^2} = K_2 dt$ .

Integration yields  $\frac{1}{a - x} = K_2 t + \text{constant}$ . When  $t = 0$ ,  $x = 0$ , so that the constant =  $1/a$  and the integrated rate now is

$$\frac{x}{a(a - x)} = K_2 t$$

In this case, let  $x = C_0 - C_t$ , and  $a = C_0$ ,  $a - x = C_t$  then  $\frac{C_0 - C_t}{C_0 C_t} = K_2 t$

Aerosols, like most colloidal forms of matter, are essentially unstable, and will usually disappear with the passage of time either by evaporation or precipitation. Evaporation will occur if the substance of



TABLE I  
RELATIONSHIP BETWEEN WEIGHT CONCENTRATION AND TIME

$C_o$ mg/100 ml	t min	$C_t$ mg/100 ml	$C_o - C_t$	$\frac{C_o - C_t}{C_o}$	$\frac{C_o - C_t}{C_o C_t}$
o 0.245	1	0.198	0.047	0.192	0.970
	10	0.130	0.115	0.470	3.620
	25	0.095	0.150	0.614	6.450
	50	0.058	0.185	0.763	13.200
x 0.492	1	0.412	0.080	0.163	0.396
	10	0.195	0.297	0.605	3.100
	25	0.110	0.382	0.777	7.060
	50	0.071	0.421	0.858	12.050
o 0.556	1	0.458	0.098	0.176	0.385
	10	0.212	0.344	0.620	2.920
	25	0.114	0.442	0.794	6.950
	50	0.070	0.480	0.864	12.400



Figure 3(a)

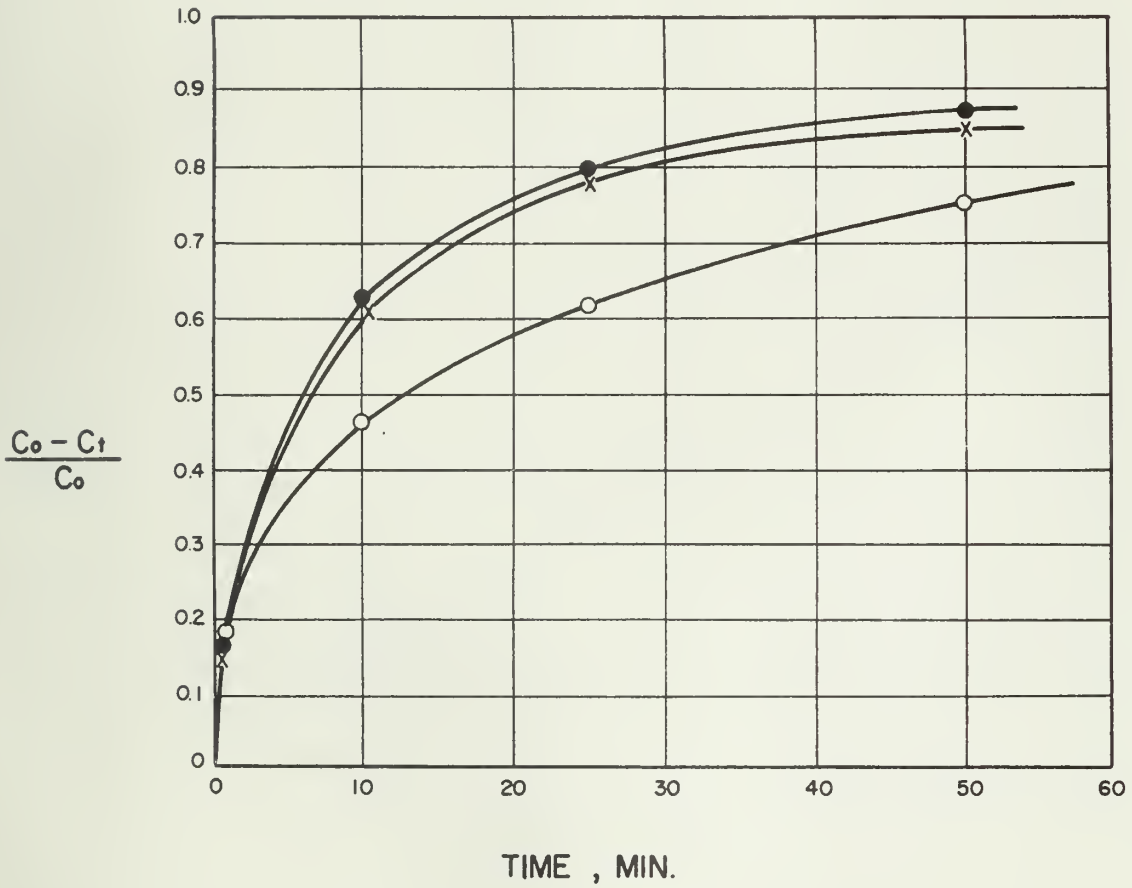
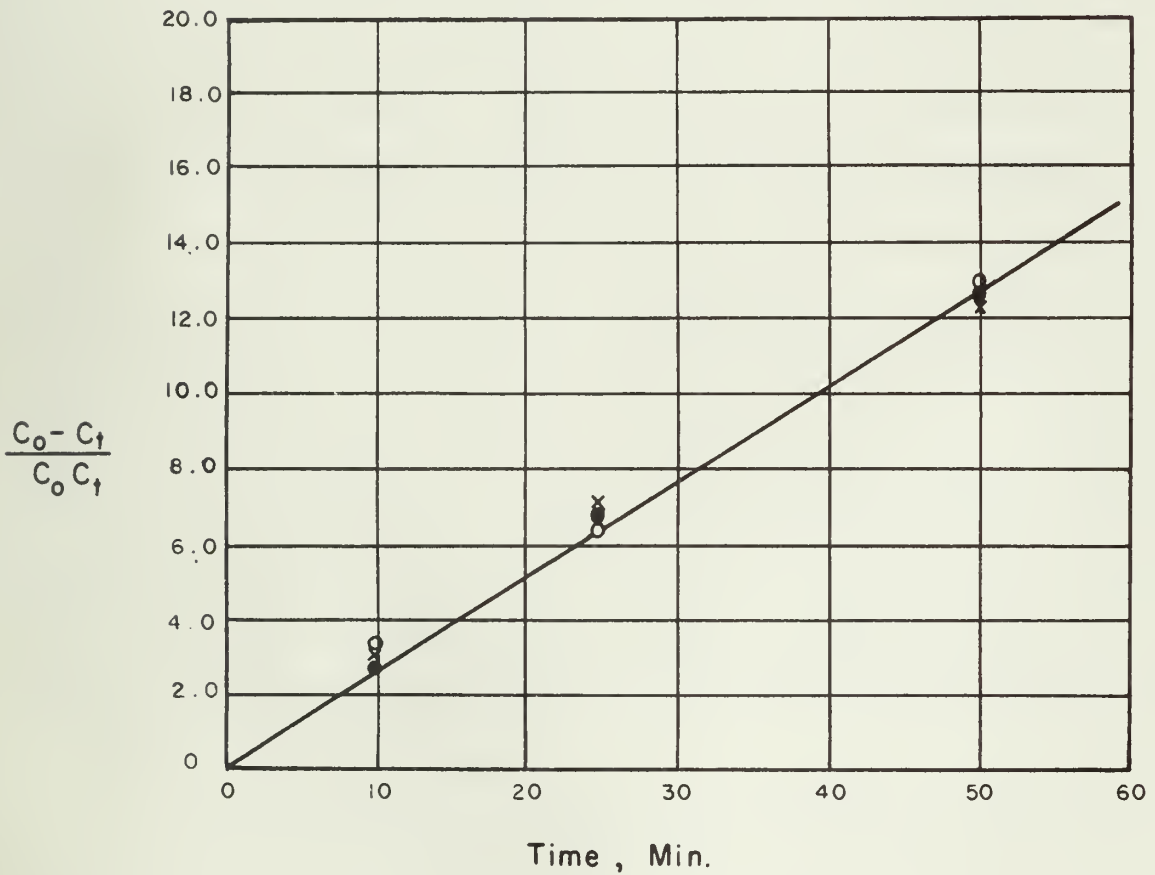
RELATIONSHIP BETWEEN WEIGHT CONCENTRATION  
AND TIME ( FIRST ORDER REACTION )



Figure 3(b)

RELATIONSHIP BETWEEN WEIGHT CONCENTRATION AND TIME  
(SECOND ORDER REACTION)







which an aerosol is composed has an appreciable vapor pressure at room temperature. The vapor pressure of a small drop is larger than that from a larger mass of substance, but this effect is not important for a drop radius greater than  $0.01 \mu$  (16).

Precipitation may occur as a result of diffusion or settling. Aerosols of large particles disappear by settling, and those of very small particles disappear by diffusion. Settling ordinarily accounts for the precipitation of aerosols of radius  $0.5 \mu$  or greater. In this experiment particle sizes are  $0.5 \mu$  or greater, therefore, diffusion is not important for the instability of aerosols. According to Stokes<sup>1</sup> law a particle falls with a steady velocity which is proportional to the square of the radius.

As the particles come into contact with each other they coalesce, form large particles, or flocculate and precipitate more rapidly than the original particles. A very simple way of increasing the coagulation rate of a particulate cloud is to make the air turbulent by mixing it. Eddies and swirls are then formed and the velocity of the particles relative to each other becomes greater. The chance of collision of particles with one another must therefore be increased, and the observed rate of coagulation will increase. On the other hand the mixing will increase the attachment of aerosols on the wall and on the stirrer.

Nessler's reagent was used to check the effect of gravitational settling upon the decrease of the mass of the aerosol as follows: After 20 minutes stirring, the weight of ammonium chloride on the wall, on the stirrer, and on the bottom of the cloud chamber respectively was found to be in the ratio 3 : 3 : 94. Therefore, we can assume the main factor in the decrease of weight concentration depends on gravitational settling. The number of particles settling is given as follows:



$$-\frac{dn}{dt} = \frac{n \cdot v}{H} \text{-----} \quad (1)$$

where

V: Stokes' velocity (cm/min) of fall for a given particle size and density.

H: The effective height of the box

There are two kinds of coagulation in the smoke box; (1) in the air flow the coagulation occurs because of the Brownian motion, and (2) the difference of speed and direction between the small particles and the big particles which moved out of the air flow, results in collisions and coagulation. When  $n$  equals the number of particles per cc in the smoke box, we can derive two equations from the above two reasons.

$$(1) \text{ Brownian motion: } -\frac{dn}{dt} = K'n \text{-----} \quad (2)$$

(2) Collision between small particles and big particles:

$$-\frac{dn}{dt} = K'' a(1 - a) n \text{-----} \quad (3)$$

Where  $a$ : rate of coagulation

Then the rate of decreasing particles is expressed from the summation of equations (1), (2), and (3).

$$-\frac{dn}{dt} = \frac{n \cdot v}{H} + K'n + K'' (1 - a)n \text{---} \quad (4)$$

In equation (4),  $\frac{n \cdot v}{H}$  and  $K'n$  are independent of stirring and  $K''a(1 - a)n$  is affected by stirring.

The result of the experiment is a second order equation; and equation (4), which is derived theoretically, also shows a second order reaction. The coagulation of aerosols resembles a bimolecular mechanism, and is described by Smoluchowski's equation for a second order reaction (17).



## (C) Calculation of Particle Size

The settling rate of a monodispersed aerosol in a closed chamber under turbulent conditions (stirred settling) is an exponential function of the concentration, which can be defined as (18),

$$-\frac{dn}{dt} = \frac{n \cdot v}{H} \text{-----} \quad (5)$$

or

$$\frac{n}{n_0} = e^{-v \cdot t/H} \text{-----} \quad (6)$$

where

$n$ : the concentration at time  $t$ .

$n_0$ : the concentration at time zero.

$v$ : Stokes' velocity (cm/min) of fall for a given particle size and density ( $\text{g/cm}^3$ ).

$H$ : the effective height of the chamber.

Equation (2) is applied by Dimmick (19) to find particle size from the light scatter decay. The logarithm of the concentration plotted against time results in a straight line, and the same slope is obtained whether  $n$  is in terms of number, geometric area, or volume (mass). The slope is defined in terms of the half-life ( $L_{1/2}$ ), a procedure preferred because equation (6) can be transformed to the linear equation:  $0.692 = \frac{v \cdot L_{1/2}}{H}$ . By Stokes' law,  $v = 7.2 \times 10^7 \rho r^2$ , where  $\rho$  is the specific gravity (density) and  $r$  is the particle radius. For convenience a nomograph was made, which relates particle size and density to half-life for a chamber height of 50 cm (Table 2 and Figure 4).

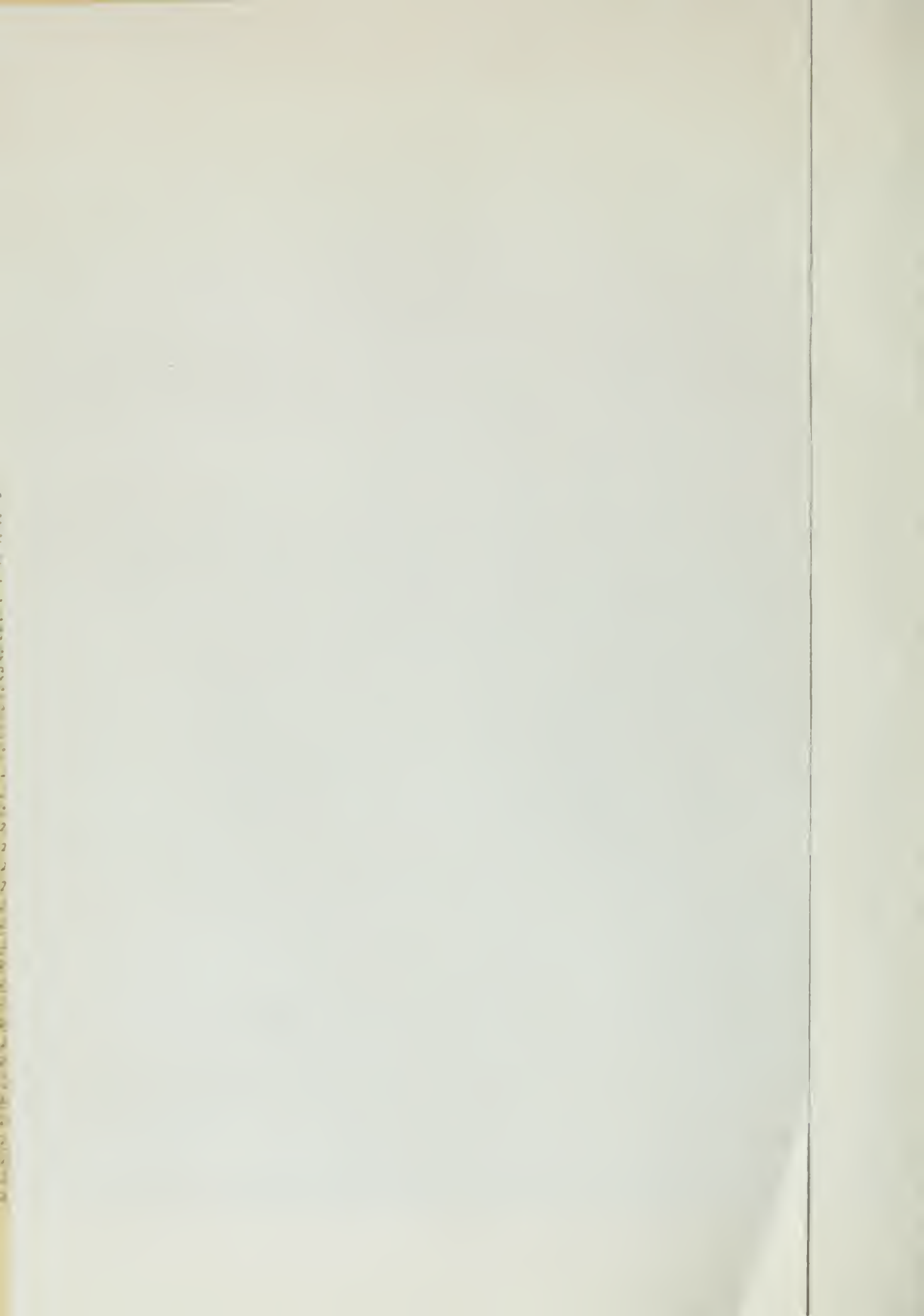
The fallout of polydispersed aerosols is more complex (20). A reasonable estimate of particle size may be obtained, however, in the following manner.







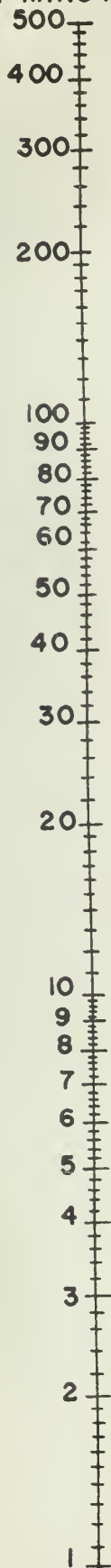




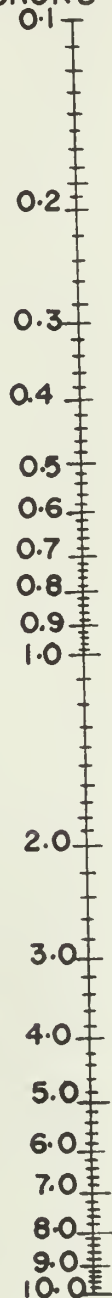


# NOMOGRAPH FOR USE IN CALCULATING STOKESIAN RADIUS FROM HALF-LIFE DATA (CHAMBER HEIGHT 50 CM)

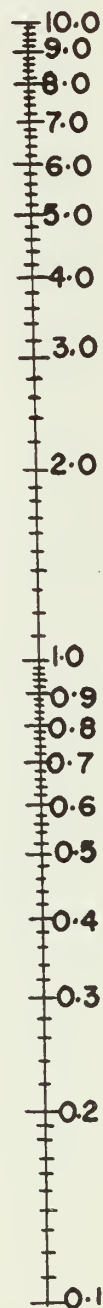
HALF-LIFE  
IN MINUTES



PARTICLE SIZE  
IN MICRONS (RADIUS)



DENSITY, GM/CC.





As the light scatter decay method is described in an earlier paper by the author, construct a light scatter decay curve and replot on semilog paper <sup>(21)</sup>. Determine a final exponential slope from the straight portion of the curve which occurs during the later lifetime of the aerosol when most of the larger particles have fallen out.

Values on this slope, at about ten minute intervals, are then subtracted arithmetically from equivalent values on the overall decay curve, and the result is plotted on the same scale to yield a second curve line representative of the aerosol less the smallest particles. This second curve is treated as the first; a final slope is determined and subtracted from the second to yield a third, etc. The result is a series of exponential slopes, each representative of an arbitrary fraction of the total decay curve, and each having a point at zero time representative of the amount of the light scatter area contributed by that fraction. The particle size of each fraction can be determined from the half-life.

The light scatter area contributed by each fraction at zero time is (approximately) a function of the square of the radius of the particles, whereas the mass is a function of the cube of the radius. Multiplying the percent of the light scatter area of each fraction, by its respective size yields the relative mass value of that fraction. These weighted mass values are then totaled, the mass percent calculated, and the cumulative mass percent plotted on log probability paper to obtain the median mass radius.

For the purpose of this discussion the size distribution of the particles is considered to follow the log probability law and the average radius of the particles, based on either linear radius, cross-sectional area, or volume (mass) are as defined by  $r_1$ ,  $r_2$ , and  $r_3$ , respectively. The general expression for any average radius whether having a real or hypothetical meaning is given by:



$$r_m = (\sum n_r r^m / \sum n_r)^{\frac{1}{m}} \text{-----} (7)$$

Where  $m$  may have the value 1, 2, 3, etc. and  $n_r$  is the number of particles having a radius  $r$ .

Initially at zero time the total cross-sectional area of particles per cubic centimeter of aerosol is given by,

$$C_0 = \pi n_0 r^2 \text{-----} (8)$$

Where  $n_0$  is the total initial number. Similarly the initial mass concentration is,

$$M_0 = \frac{4\pi}{3} n_0 \rho r^3 \text{-----} (9)$$

Where  $\rho$  is the density of the particles.

Due to stirred settling the cross-section per cubic centimeter and mass decrease according to equation (6) and may be integrated over the whole particle size range given at time  $t$  where  $\sigma$  is the standard deviation,

$$C_t = \pi \int_0^{\infty} r^2 n_r e^{-\frac{v t}{H} \sigma \ln 2r} \text{-----} (10)$$

$$M_t = \frac{4\pi}{3} \int_0^{\infty} r^3 n_r e^{-\frac{v t}{H} \sigma \ln 2r} \text{-----} (11)$$

Where  $v$  is the terminal settling velocity of a particle of radius  $r$ .

If use is made of the simple Stokes' law,  $v$  is found to be equal to  $7.2 \times 10^7 \rho r^2$  cm/mIn, where  $r$  is expressed in cm.

Taking logarithms of  $C$  to base 10, differentiating with respect to  $t$  and substituting the value  $v$ , equation (10) yields,

$$-\frac{d(\ln C_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot \frac{\int_0^{\infty} r^4 n_r e^{-\frac{v t}{H} \sigma \ln r} \text{---}}{\int_0^{\infty} r^2 n_r e^{-\frac{v t}{H} \sigma \ln r} \text{---}} \text{---} (12)$$



when  $t$  is small compared with  $H/v$ , equation (12) becomes

$$-\frac{d(\ln C_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot \frac{\int_0^\infty r^4 n_r \sigma \ln r}{\int_0^\infty r^2 n_r \sigma \ln r} \quad (13)$$

or

$$-\frac{d(\ln C_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot \frac{r_4^4}{r_2^2} \quad (14)$$

Similarly it may be shown that

$$-\frac{d(\ln M_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot \frac{r_5^5}{r_3^3} \quad (15)$$

Hatch and Choate (22) integrated the log probability distribution expression to derive the general equation

$$\ln r_m^p = p \ln r_g + 2.303 \frac{p \cdot m}{2} \cdot \ln^2 \sigma_g \quad (16)$$

Where  $r_g$  is the geometric mean radius and  $\sigma_g$  is the geometric standard deviation. This equation can be applied to transform equations (14) and (15).

$$-\frac{d(\ln C_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot r_6^2 \quad (17)$$

and

$$-\frac{d(\ln M_t)}{dt} = 3.13 \times 10^7 \cdot \frac{\rho}{H} \cdot r_8^2 \quad (18)$$

From Hatch (23) it may be seen that  $r_6$  can be identified as the median mass radius. By introducing the values of  $r_6$  and  $r_8$  in equation (16),  $\sigma_g$  and  $r_g$  may be calculated and hence the number size-distribution can be deduced from the log probability law.





## IV. RESULTS

When the decrease of an aerosol mass (weight concentration) in the cloud chamber, with stirring, was studied with Nessler's reaction, the rate of decrease was found to be a second order reaction. This means that the decrease of weight concentration of an aerosol in the cloud chamber was mainly controlled by the coagulation because the theoretical equation of coagulation of an aerosol is second order and resembles a bimolecular reaction. The coagulation of an aerosol described by Smoluchowski is a second order reaction. He applied a constant value to this equation which depends on the assumption that the collision efficiency of an aerosol is 100%. Therefore, the effect of foreign vapors on the coagulation of aerosols was studied to determine whether the collision efficiency between the particles of an aerosol was 100% or not.

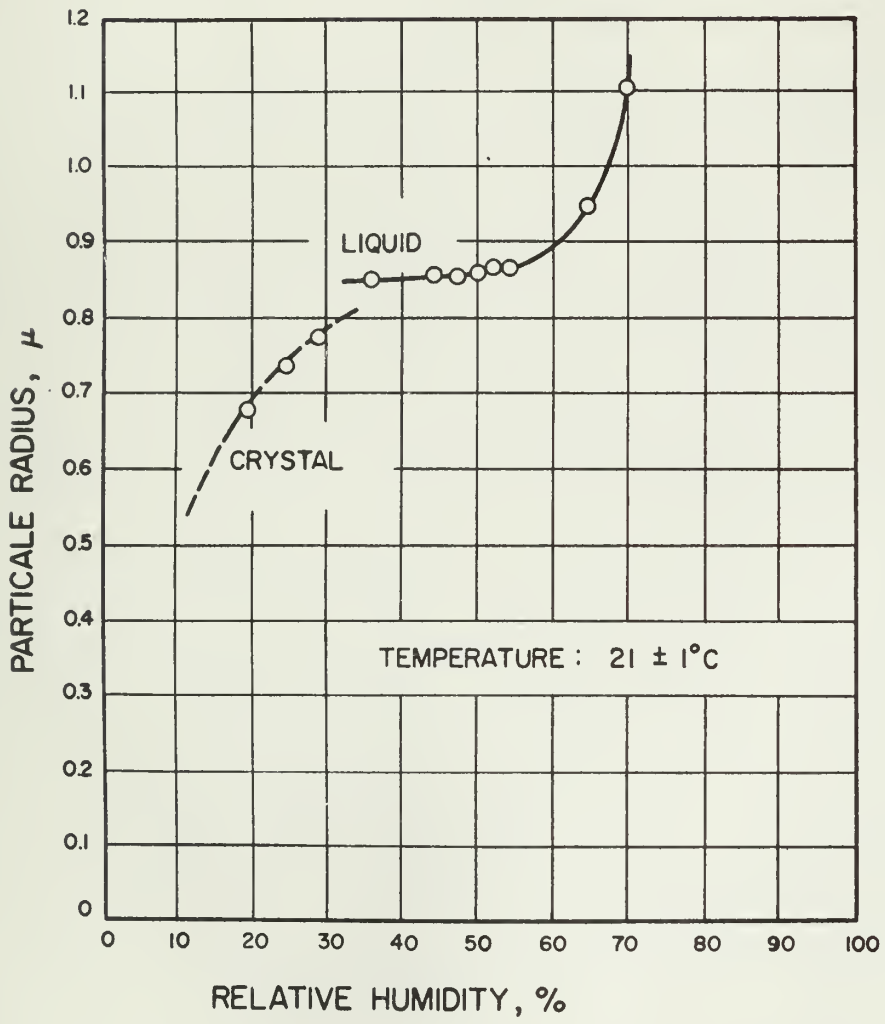
Water vapor is seen to decrease slightly the stability of an aerosol of an ammonium chloride solution when the relative humidity is 50% or less. If the relative humidity is higher than 55%, great aggregation is found experimentally. This effect is probably due to the fact that substances which lower the vapor pressure of the aerosol constituent can significantly increase the collision probability by the removal of the vapor cushion surrounding a particle. That is, ammonium chloride, being hygroscopic, causes a lower number of water molecules near each particle of an ammonium chloride solution than in the main body of the air, which results in a somewhat reduced pressure in the vicinity of the particles and makes it somewhat easier for two particles to collide. Figure 5 shows the relationship between the relative humidity and the size of an aerosol of ammonium chloride solution.

An expression for the equilibrium vapor pressure at the surface of a droplet of solution was derived by Mason <sup>(24)</sup>. Consider a solution droplet,



Figure 5

### RELATIONSHIP BETWEEN RELATIVE HUMIDITY AND PARTICLE SIZE OF AMMONIUM CHLORIDE





radius  $r$ , at the surface of which the vapor pressure  $P_r'$ , to be in equilibrium with an atmosphere in contact with a plane water surface whose equilibrium vapor pressure is  $P_{\infty}$ . If an element mass  $dm$  of water is transferred from the droplet to the plane water surface, the resulting decrease in the free energy of the solution will be:

$$\Delta\phi = \gamma' dA - P dV = \frac{2 \gamma' dm}{\rho_L' r} - \frac{P dm}{\rho_L'} \quad \text{-----} \quad (19)$$

where  $dA = (8 \cdot \pi \cdot r \cdot dr)$  and  $dV$  are the changes in the surface area and the volume of the droplet,  $\gamma'$ ,  $\rho_L'$ , and  $P$  are respectively the surface tension, density, and osmotic pressure of the solution. Alternatively, the decrease of free energy is given by the work gained in evaporating the mass  $dm$  of water at pressure  $P_r'$ , expanding the vapor to the lower pressure  $P_{\infty}$  and condensing it at pressure  $P_{\infty}$ , the whole process being carried out reversely and isothermally. Assuming the water vapor to behave as an ideal gas, then

$$\Delta\phi = dm \frac{R T}{M} \ln \frac{P_r'}{P_{\infty}} \quad \text{-----} \quad (20)$$

where  $R$  is the universal gas constant,  $T$  the temperature, and  $M$  the molecular weight of water. Equating the two expressions for  $\Delta\phi$  gives

$$\ln \frac{P_r'}{P_{\infty}} = \frac{2 \gamma' M}{\rho_L' R T r} - \frac{P M}{\rho_L' R T} \quad \text{-----} \quad (21)$$

For solutions whose density vary linearly with concentration (this is very nearly true for  $\text{NaCl}$ ,  $\text{MgCl}_2$ ,  $\text{NH}_4\text{Cl}$ , etc.),  $P = \frac{R T \rho_L}{M} \cdot \ln \left( 1 + i \frac{n_1}{n_2} \right)$

where  $n_1$  is the number of moles of solute dissolved in  $n_2$  moles of water,  $\rho_L$  is the density of water, and  $i$  is Van't Hoff's factors which depend upon the chemical nature and the degree of dissociation (i.e., on the concentration) of the solute.

When the solution droplet is in equilibrium with the surrounding atmosphere, with its surface temperature equal to that of the air  $P_r'$  must



equal the partial pressure of the water vapor, so  $H/100 = P'_r/P_\infty$ , where  $H$  is the relative humidity of the air. Thus the condition for equilibrium becomes:

$$\ln \frac{P'_r}{P_\infty} = \frac{2 \gamma' M}{\rho'_L R T r} - \frac{P M}{\rho'_L R T} = \frac{2 \gamma' M}{\rho'_L R T r} - \frac{R T \rho_L}{M} \cdot \ln \left( 1 + i \frac{n_1}{n_2} \right) \cdot \frac{M}{\rho'_L R T}$$

$$\ln \frac{P'_r}{P_\infty} = \ln \frac{H}{100} = \frac{2 \gamma' M}{\rho'_L R T r} - \frac{\rho_L}{\rho'_L} \cdot \ln \left( 1 + i \frac{n_1}{n_2} \right) \quad \text{----- (22)}$$

For example:

$$\ln \frac{H}{100} = \frac{2 \gamma' M}{\rho'_L R T r} - \frac{\rho_L}{\rho'_L} \cdot \ln \left( 1 + i \frac{n_1}{n_2} \right)$$

$$\gamma' = 73 \text{ erg/cm}^2, \quad m = 18 \text{ g/mole}, \quad R = 8.3 \times 10^7 \text{ erg/deg. mole},$$

$$T = 293^\circ\text{K}, \quad \rho'_L = \frac{50}{100} \times 1.53 + \frac{50}{100} \times 1.00 = 1.26 \text{ g/cm}^3, \quad \rho_L = 1.00 \text{ g/cm}^3$$

(assuming the concentration of particles is a 50% solution)

$$r = 1 \times 10^{-4} \text{ cm}, \quad i = 2.7, \quad \frac{n_1}{n_2} = \frac{50/53.5}{50/18} = 0.3$$

$$\ln \frac{H}{100} = \frac{2 \times 73 \times 18}{1.26 \times 8.314 \times 10^7 \times 293 \times 1 \times 10^{-4}} - \frac{1 \times \ln (1 + 2.7 \times 0.3)}{1.26}$$

$$= \frac{-\ln 1.81}{1.26} = \frac{-0.59}{1.26} = -0.47$$

$$\frac{H}{100} = 0.625$$

$$H = 62.5\%$$

Results obtained experimentally by others for the same system show the following: Smirnov and Solntseva <sup>(5)</sup> reported the effectiveness of water vapor as well as butyric acid as aggregants for an ammonium chloride aerosol, but Samokhvalov and Kozhukhova <sup>(6)</sup> state that low concentration of either water or octyl alcohol act as a stabilizer, rather than as an aggregant for ammonium chloride aerosols. Dalla Valle, Orr and Hinkle <sup>(7)</sup>, however, report that water vapor produces the greatest aggregation in such aerosols.

Many experiments have been carried out on the influence of foreign vapors on coagulation of aerosols, but the literature reveals many cases of apparent disagreement in this field. Some workers have reported a stabilizing





effect of a particular vapor on an aerosol; others found no effect or even decreased stability, for the same system. One of the main reasons for this disagreement is that in most of the experiments the sample of aerosol was taken out of the cloud chamber (or smoke box) through a tube, thereby changing the characteristics of the aerosol, because the large particles dropped out as they passed through the tube. The light scatter decay method possesses the advantage that it may be applied to the study of an aerosol without changing the characteristics of the aerosol, because this technique measures directly the change of light intensity of a Tyndall beam as an aerosol settles under turbulent conditions in a cloud chamber. Another reason for the disagreement is the fact that the coagulation rate was not usually determined directly, by measuring the rate of diminution of particle concentration, but was inferred from the settling of the aggregates.

Green and Lane <sup>(25)</sup> concluded that a change in the coagulation rate in the presence of a foreign vapor is possible only in the case of aerosols of solid particles, and that the reason for a variation is not an increase or decrease in the effectiveness of the collisions between the particles, but is due to a change in shape of the aggregates formed.

These experiments herein studied show that a change in the coagulation rate in the presence of a foreign vapor is also possible with aerosols of liquid particles, and that the vapor cushion surrounding the suspended ammonium chloride particles is one of the important factors in the coagulation of ammonium chloride.



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## Part 2. SHORT STORAGE STUDY ON THE VIABILITY OF AIRBORNE BACTERIA

I. Hayakawa  
C. P. Poon

### I. INTRODUCTION

#### (a) Airborne Infection

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 airborn micro-organisms and methods of air hygiene such as disinfection by ultraviolet light and by chemical agents have been applied increasingly to their control.

One of the most important methods of spread of certain microbial diseases 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  $\mu$  in diameter, of saliva and other secretions, some containing micro-organisms, are expelled into the air with considerable velocities <sup>(1)</sup>. The larger droplets are deposited on nearby objectives 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 content of micro-organism suspended in the air, called "droplet-nuclei". Such "droplet-nuclei" may remain airborne for long periods of time and be carried long distances.

It is therefore evident that the mechanical transmission of airborne infection depends entirely on the ability of the bacteria to survive while staying 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 Micro-organisms

Factors governing the viability of airborne bacteria are numerous. Among them are temperature; relative humidity (R.H.); particle size, the presence and absence of toxic material, salts and growth medium. Studies in this area have been conducted over the past two decades. The effects of R.H. and temperature on the airborne cells has formed the greater part of these studies, but conclusions drawn from these studies differ widely. Some researchers have stated that the rate of death of the airborne cell is greatest at high R.H. levels (2, 3) while others have stated that the greatest rate of death occurs at intermediate R.H. levels (4, 5, 6). Some reported, on the other hand, that the lowest R.H. levels are most lethal (7, 8). More recently, a modern technique of using radioactive cells in aerobiological studies (8, 9) provides a means to distinguish between real and apparent death due respectively to loss of viability of the airborne cells and particulate settling. Some experiments were conducted with different rates of flow in the storage systems and consequently apparent environments were different. The situation is further complicated when airborne cells of different growth phases, in the presence of growth medium or without the growth medium, in the droplets atomized are employed for study. It is obvious that any attempt to compare the data among the workers would be valueless if all existing factors are not taken into consideration.

In order to eliminate the complicated situation, experiments were conducted in different series. The first one, using distilled water in bacterial suspensions prepared for generating aerosols, served as a control since in this case the only controlling factors were temperature and R.H. Subsequent



series were conducted, by adding one or more controlling factors, with or without eliminating some of the investigated factors. Experiments arranged in this way would show the effect of each of the factors on the airborne bacteria and the role it played in the mechanisms of the death of airborne bacteria.

(c) Organism 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 facilities not available in the laboratory for handling pathogens, therefore, it was determined to use non-pathogens in order to eliminate any health hazard possibly occurring to the laboratory personnel.

E. coli has the ability to grow in a defined medium. Its growth is reasonably rapid, and it is easy to handle. In addition, it has been studied in other laboratories. Such studies provide a wealth of information and permit a comparison of results.

Another virtue of E. coli which makes it more convenient for study is that its membrane is sufficiently permeable<sup>(10)</sup>. The metabolically active centers of the cells are in intimate contact with the environment. This readily suggested the idea of rapid evaporation of water content of the cells as the cause of death which was the main part of the study in the author's work.

(d) Purpose of the Study

It is the purpose of this study to use E. coli to investigate the effects of different factors on the viability of airborne bacteria and the mechanism of the death. Radioactive phosphorous  $P^{32}$  was used to label the bacteria in order to differentiate the physical loss in the storage chambers and the actual death of the organisms.



This part of the study, including the short storage study in which a short storage chamber was used to provide storage time ranging from one-half second to four and a half seconds, was primarily for investigating the immediate effects of temperature, R.H., and characteristics of the bacterial suspension on the death of airborne bacteria which was proved, from the present study, to be a result of rapid water evaporation from the cellular material within the bacterial cells. Other factors which do not have immediate effects were not studied.

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 author's efforts in a limited time to study on one single species of bacteria. The outcome of which, therefore, can not be applied to all different kinds of bacteria, but it is felt that the result gives a general significance of the effects on airborne bacteria under different environments.



## II. THEORETICAL CONSIDERATION

### (a) The Effect of Relative Humidity on the Viability of Airborne Bacteria

When a bacteria culture is suspended in distilled water as a spray solution, the osmotic pressure of distilled water is not an important factor in the viability of bacteria because bacteria are remarkably resistant to changes in osmotic pressure and are not disturbed or plasmolyzed by suspension in hypotonic or hypertonic solutions. By taking the osmotic effect out of consideration, the only factors causing damage to the airborne bacteria cells sprayed from distilled water and stored for merely a short time are temperature and R.H. since no extrinsic effect by foreign matter is present. Furthermore when temperature in the storage chamber is kept within a range in which the bacteria will maintain life, the heat killing in the mechanism of viable decay is ruled out.

When R.H. is taken into consideration, it is readily suggested that humidity is closely connected to hydration and dehydration processes, or an exchange of water molecules in and out of the cell membrane. Thus R.H. and temperature, which in turn affects the R.H., have a combined effect on the death of bacteria by removing water out of or in the bacterial cells and the rate of death is governed by the changes of temperature and R.H. in the environment to which the bacterial cells are exposed. The faster the rate of evaporation of water from aerosol particles is, the faster the rate of bacterial death is anticipated.

### (b) Protein Structure and Binding Water

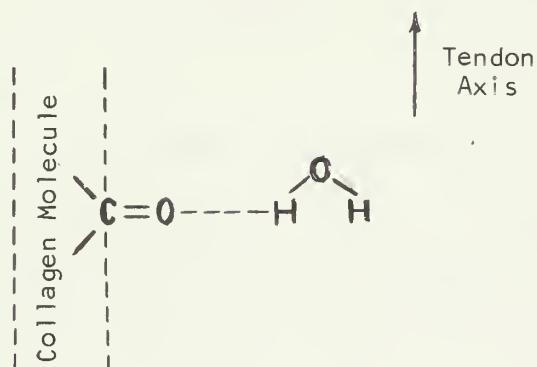
The primary structure of the protein molecule is the peptide chain built up by L-amino acids. The works of Mellon et al. (11, 12, 13) have suggested that the peptide groups can participate in water binding. Perhaps the most evident proof of the presence of water bonded to protein molecules is





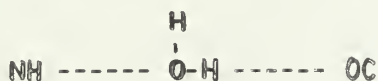
that reported by Fraser and Macrae <sup>(14)</sup>. According to their work, investigations of the infra-red spectrum of collagen in the  $2 \mu$  region showed that hydrated collagen contains a proportion of water molecules which are preferentially oriented with respect to the polypeptide chains.

The preferred orientation was suggested by Fraser and Macrae as follows:

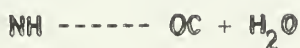


The configuration of the polypeptide chain in collagen is believed to be such that the CO linkages are approximately normal in direction to the collagen chain axis protruding out from the triple chain molecule and so are not available for intra-molecular hydrogen bonding as mentioned by Crick and Kendrew <sup>(15)</sup>. 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 <sup>(16)</sup>, 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 very possible that water molecules are attached to the protein at various sites. The



bonds at various sites have different bond strength, some of them being loose bonds and some of them very strong bonds which would require a great amount of energy in order to break them. Bonding sites of water molecules could also determine its relationship to cellular death in such a way that water molecules could be attached to sites not vital to cellular stability and could also be bonded to sites vital to cellular stability.

### (C) Denature of Protein

Among others, one phenomenon in the denature of protein molecules is to remove bonded water molecules from the protein. Since water molecules have been shown to be an integral part of protein molecules, "dehydration" of the protein results in its inactivation which would finally cause the death of the bacteria.

In fact the idea of removal of water molecules within cells causing rapid death is not new. Scott <sup>(17)</sup> stated that the removal of the most firmly held water molecules results in some loss of bacterial stability. Ferry, Brown and Damon <sup>(18)</sup> 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. In the case of stationary evaporation of a spherical droplet, Maxwell expressed the rate of diffusion of the vapour of the droplet across any spherical surface with radius  $r$  by the following equation:

$$I = \frac{dm}{dt} = 4\pi r D (C_{\infty} - C) \quad (1)$$



Where  $C_{\infty}$  is the concentration of vapour at infinite distance from the drop with radius  $r$ , and  $C$  its density or 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.  $D$  is the diffusion coefficient of the vapor and  $m$  the mass of a diffusing droplet.

If we assume that the vapour obeys the ideal gas laws and if we express its concentration as the partial vapour pressure  $p$ , then

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

where  $R$  is the universal gas constant and  $T$  the absolute temperature. Substituted into Maxwell's equation, we have

$$-\frac{dm}{dt} = \frac{4\pi r D M (P_0 - P_{\infty})}{R T} \quad (2)$$

For very small droplets, the evaporation is proportional to changes of surface area, the previous equation can be transformed into:

$$-\frac{dA}{dt} = \frac{8\pi D M}{R T d} (P_0 - P_{\infty}) \quad (3)$$

with  $d$  the density of the liquid drop.

Strictly speaking, the evaporation of a droplet can not be a stationary process since the radius and hence the rate of evaporation is constantly decreasing. But as shown by Fuchs <sup>(19)</sup> when  $C \ll d$ , the evaporation can be regarded as quasi-stationary i.e. the radius can be regarded as a constant value.

Fuchs had another approach for developing an equation for water evaporation as reviewed by Green and Lane <sup>(20)</sup>. He considered the diffusion process as starting not directly at the surface of the evaporating sphere but at a distance of  $\Delta$  apart from the droplet surface. In other words, the evaporation starts from the surface of an enveloping sphere of radius  $r + \Delta$ , where  $\Delta$  is of the order of the mean free path of the diffusing molecules. Very few molecules



will then be present in the spherical shell of thickness  $\Delta$  which represents the distance traveled by an evaporating molecule before it collides with a gas molecule.  $\Delta$  can be calculated from the formula

$$\Delta = \lambda \left( \frac{m_1 + m_2}{m_1} \right)^{\frac{1}{2}} \quad (4)$$

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

Considering the equilibrium condition where the diffusing molecules arriving at the surface,  $r + \Delta$  away from the center of the droplet, at a rate equal to the rate at which molecules leave the surface by diffusion, Fuchs finally developed the following equation:

$$-\frac{dA}{dt} = \frac{8 \pi M}{R T d} (P_0 - P_{\infty}) \cdot r \sqrt{\alpha} \quad \text{for very small } r, \quad (5)$$

where  $\alpha$  = evaporation coefficient and  $\sqrt{\phantom{x}} = (K T / 2m_2)^{\frac{1}{2}}$ ,  $K$  being the gas constant per molecule.

However, when water is evaporated, the heat is removed from the drop and the surface temperature on the water droplet is lower. An equilibrium temperature  $\theta$  will be reached later which 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 would cause a great change in the vapor pressure of the droplet surface.

The equilibrium temperature  $\theta$  for an evaporating droplet was given by Johnson (21) as:

$$\theta = \frac{L M D}{K R T} \frac{(f \cdot P_T - P_{\theta})}{(D/r \sqrt{\alpha + 1})} + T \quad (6)$$

in which the  $\theta$  is the equilibrium temperature in absolute degrees,





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

K the thermal conductivity of the air, 0.014 Btu/(cm<sup>2</sup>. sec.) (1<sup>o</sup>F/in.) or 0.000288 g. cal/ (cm<sup>2</sup>. sec.) (1<sup>o</sup>C/cm),

f the relative humidity expressed as a fraction,

P<sub>T</sub> the vapor pressure of the liquid at ambient absolute temperature.

P<sub>o</sub> the vapor pressure of the liquid at equilibrium temperature,

D the diffusion coefficient, 0.21 + 0.0015 T<sup>o</sup>C. (22)

Equation (5) is adjusted with this temperature drop as in the following:

$$\frac{dA}{dt} = \frac{8\pi M}{R T d} r V \alpha. (f \cdot P_T - P_o) \quad (7)$$

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

The previous discussions consider only the case of evaporation of a droplet at a rest or of a droplet which has no relative velocity with the moving gas stream. When a droplet is in motion relative to the surrounding gaseous medium, whether the droplet is fixed with the gaseous stream passing by or the droplet is moving along with the gaseous stream at different velocities due to the turbulence of the flow, a wind factor f should be applied. Frossling (23) showed that the wind factor is a function of Reynold's number for the flow:

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

Kinzer and Gunn (24) showed through experiments that  $f = (1 + 0.22 F Re^{\frac{1}{2}})$  where F should be a function of Re and not a constant as suggested by Frossling.

#### (e) Evaporation of Aerosols

Bacterial aerosols are made of various substance differing in physical, chemical and biological characteristics. They are more complicated than droplets of pure substances in droplet evaporation. In water droplet evaporation, the rate 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. The first one lies in the fact that water molecules within the cell are bonded to protein molecules with hydrogen bonds at various sites on the protein molecules. 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 free water. The rate of breakage of such protein-water bonds is therefore a limiting factor regarding the water evaporation. The second factor is the presence of the cell membrane which attenuates the rate of vapor and heat transfer from within the cell to the outside environment. The cell membrane in fact, increases the thickness of the imaginary boundary shell through which a diffusing water molecule has to travel 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 or in cellular water except that the rate is much slower in the latter case. The equation expressing the rate of water evaporation should apply equally well to both cases, except that in the case of cellular water evaporation, a constant is added to the equation to account for the difference of vapor and heat transfer. Therefore we have:

$$\frac{dA}{dt} = \text{Constant} \cdot \frac{8\pi M}{R T d} r \gamma \alpha (f \cdot P_T - P_\ominus) \quad (8)$$

For pure water droplets, the constant is equal to unity. The  $\alpha$  here is a constant value for the cellular material of one particular kind of bacteria on the average, but no longer equal to 0.04 which is for the case of pure water<sup>(25)</sup>. It has been shown by Eisner, Quince and Slack that a reduction of



this evaporation coefficient  $\alpha$  by dispersing an insoluble agent in water to form insoluble monolayers on sprayed aerosols cuts down the water evaporation by a factor up to several hundred. It is easily visualized then, that the cell membrane would reduce  $\alpha$  to a much smaller value and consequently the water evaporation from within the cell is greatly reduced.

As has been described, the inactivation of bacteria starts when its protein-water bonds are broken and the "freed" water starts to evaporate. The time period for the surrounding water to evaporate to such an extent that the cellular water starts to evaporate is therefore, very important in the study of the viability of airborne cells in a very short storage time. From calculation, the water layer surrounding the bacteria evaporates toward completion within a short time. This suggests that the bonded water molecules would be broken almost immediately after the aerosols are sprayed which agrees with the immediate lethal effect on the bacteria.

(f) The Effect of Sodium Chloride on the Evaporation of Aerosols

Sodium chloride has the distinct characteristic of lowering the vapour pressure of the liquid medium in which it exists and consequently reduces the liquid evaporation. However, the reduced vapor pressure is only a few millimeters of mercury for low NaCl concentrations such as the biological saline water used in the present study. The "dehydration" effect exerted on the bacterial cells by the surrounding NaCl solution of an aerosol is more important. Since water molecules are leaving the solution at an enormous rate toward completion of evaporation, the concentration of NaCl in the solution is increasing rapidly. As a result, the dehydration takes place because of the higher osmotic pressure. Another possibility of dehydration is that at high salt concentrations, the number of charged groups contributed by the salts is enormous compared to those of protein molecules and therefore more polarizable water molecules in the protein are attracted around the salt ions.



Furthermore the dehydration of the protein water will start before the complete evaporation of water surrounding the cells. This would not happen in the case of bacterial aerosols sprayed from distilled water, because the NaCl concentration is high enough to attract the cellular water molecules before all water is evaporated.





## III. EXPERIMENTAL EQUIPMENT AND PROCEDURES

(a) Storage Chamber

The complete set up of the short storage chamber apparatus is shown in Figure 1 on the following page. The main chamber consisted of a 2 inch I.D. Pyrex glass tube. Nine sampling outlets along the upper part of the chamber were each 7 inches apart so that when the rate of flow in the chamber was controlled at 43 l/min., the storage time from one sampling outlet to the next one was half a second. Two dry bulb thermometers placed at both ends of the chamber and a wet bulb thermometer at the outlet end of the chamber served the purpose of R.H. indication. Heating tapes with silicone rubber (Sargent S-40853-2) were wrapped around the chamber for temperature control with the aid of autotransformers (Sargent S-30941). The flow leaving the chamber went through a flowmeter and a recirculation pump, then part of it was exhausted through an exhaust line controlled by a valve. The remaining portion proceeded along the recirculation line through a needle valve, for flow control in the system, a bacterial filter and a dehumidifier. It then entered the main chamber again, through a three-way opening joint through which humidity controlled air flow and aerosols also entered. One section of the tube between the three-way joint and the main chamber was interchangeable. Another piece of lucite tube of 0.875 inch inside diameter, 5.35 inches long, with five sampling outlets, was used for lower flows other than 43 l/min. These five outlets were 1.27, 1.75, 2.27, 3.08 and 4.30 inches from the inlet end and corresponded to one-half second storage of flows of 10.8, 12.7, 14.4, 17.3 and 21.6 l/min. respectively.

(b) Aerosol Generating Unit

A Nebulizer No. 40 atomizer was used for generating bacterial aerosols, along with a Devilbiss Compressor 501. The rate of flow controlled by a screw



## SHORT STORAGE CHAMBER APPARATUS

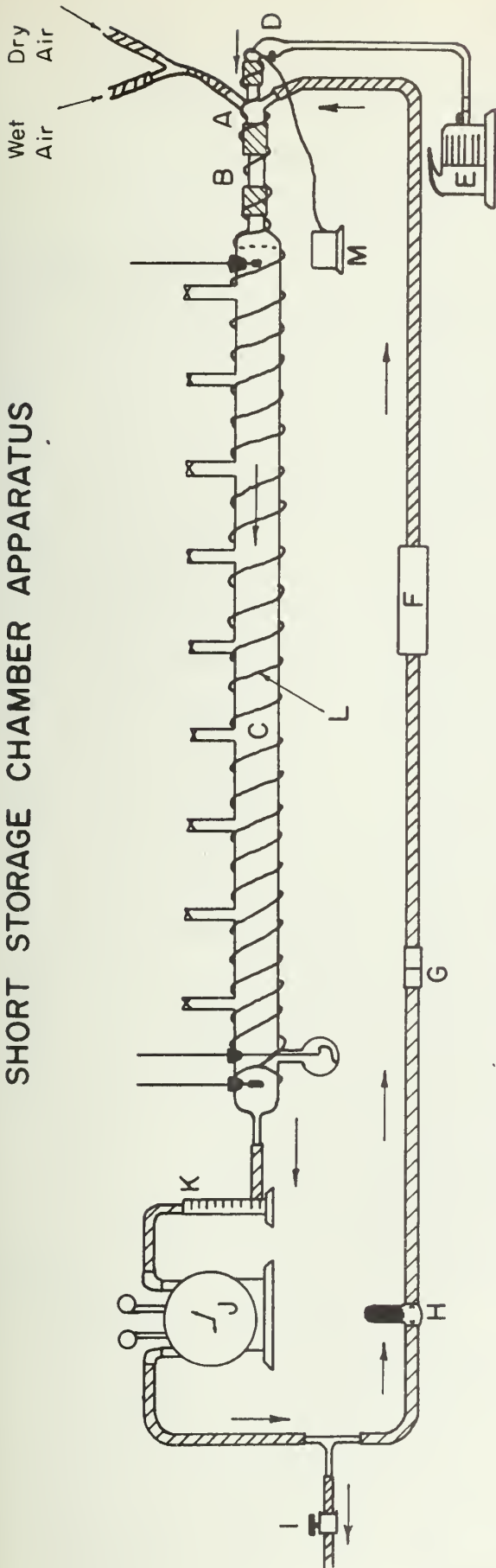


Figure 1

- 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



adjustment on the compressor unit was kept at 4.5 l/min, and 2.5 psig. The rate of atomization under this condition was found to be 0.101 ml/min. which was used throughout all experiments in the study.

(c) Sampling Unit

Graduated, all glass midget impingers (MSA midget impinger) were used with 10 ml of 0.85% saline water as collecting fluid. A Gast 0211-V36 vacuum pump was used in drawing sampling air from the storage chamber at a rate of 2.6 l/min. controlled by a needle valve in the Matheson flowmeter (Matheson Model 603).

(d) Bacterial Culture

E. coli were grown in a defined medium for all experiments:

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

E. coli cultures were harvested in the stationary growth phase throughout all experiments in the study.

(e) Experimental Procedures

E. coli cultures were harvested and washed three times by repeated centrifugation and resuspension in either one of the following solutions depending on which bacterial suspension was to be studied in the storage system: distilled water; 0.85% saline water; 5% saline water and 0.375% glycerol solution.



Then 0.1 ml of this suspension was removed and was serially diluted in a saline solution and a viable count was determined by the drop plate method using EMB agar. One ml of the same aliquot was placed in a 2-inch diameter aluminum planchet with concentric rings. The R.A. count on this suspension per millimeter was obtained by placing this planchet with the dried aliquot in a proportional counter (NMC internal proportional counter, Model DS-1A, Indianapolis) and counted for 10 min.

The suspension was then sprayed as a bacterial aerosol in the chamber by operating the aerosol generating unit. When constant temperature and R.H. was reached at the desired levels, samples at different storage times were taken from the various outlets. Viable counts and the R.A. for each sample were determined in the same way as for the spraying suspension (zero time sample).

(f) Calculation

Let the number of viable cells/0.1 ml of spray suspension =  $V_0$ , and the R.A. counts per minutes/0.1 ml of spray suspension =  $(R.A.)_0$ , the ratio  $\frac{V_0}{(R.A.)_0}$  represents the number of cells per unit radioactive count. The total cells regardless of their viability in each of the samples could be calculated as follows:

$$T_t = \frac{V_0}{(R.A.)_0} \times (R.A.)_t$$

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

$(R.A.)_t$  = R.A. counts per minute/0.1 ml of collecting fluid.

The total cells ( $T_t$ ) are comprised of living and dead cells. The number of viable cells per 0.1 ml of collecting fluid can be determined by the drop





plate counts, as  $V_t$ . The difference between  $T_t$  and  $V_t$  was the actual death of bacteria.  $\frac{V_t}{T_t} \times 100 =$  percentage of survival after storage time  $t$ , or

$1 - \frac{V_t}{T_t} \times 100 =$  percentage of death of bacteria after storage time  $t$ .



#### IV. DETERMINATION OF THE SIZE OF AEROSOLS

The size of aerosol particles sprayed from an atomizer depends on a number of things. Among them are type of atomizer used, salt concentration of the generating solution (26, 27), and jet location relative to the surface of the generating solution in the atomizer (28). In the present study a bacterial suspension of a concentration of about  $2.2 \times 10^9$  bacteria/ml was prepared and aerosols were generated with a Devilbiss Nebulizer No. 40 under 2.5 psig and 4.5 l/min. of air flow.

The initial size of aerosol particles leaving the atomizer can not be determined directly because water is readily evaporated from the aerosols which makes it impossible to observe directly or indirectly their original sizes if any lapsed time is allowed before the observation. The only means is through indirect calculation.

A simple experiment was conducted by spraying the aerosols and allowing them to deposit 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 was counted under a microscope with high power magnification and the average number of bacteria in an aerosol was calculated. By dividing this average figure with the bacteria concentration of the sprayed solution, the volume of an average aerosol was obtained and consequently the diameter was determined. With a concentration of  $2.2 \times 10^9$  bacteria/ml, the diameter of an average size aerosol was determined in this way to be approximately 13  $\mu$ .

When the aerosol evaporates, only its content of bacteria is left which would be much smaller than 13  $\mu$ . The present study shows a 13  $\mu$  size aerosol when dried gave a bacterial mass of about 0.9  $\mu$  diameter (equivalent diameter of the bacterial mass by assuming each individual E. coli cell being the size of 0.5  $\mu$  x 1.0  $\mu$ ).



## V. RESULTS

(a) Rate of Death

Results in the present study showed a high percentage of death of the airborne bacteria immediately following the formation of the bacteria aerosols. A large number of cells were found to die off within the first half second storage time while the subsequent death during the next 4 seconds was relatively slow. This phenomenon occurred in every experiment regardless of the type of suspension of the bacterial aerosols (bacteria suspended in different solutions prepared for aerosol generation).

The death rate of airborne bacteria could be expressed as a first order equation as follows:

$$k = \frac{1}{t} \ln \frac{N_0}{N_t} \quad (9)$$

in which  $N_0$  is the original number of living bacteria and  $N_t$  the number of living bacteria at the sampling time  $t$ . Since the death rate changed abruptly after one half second storage time, it was necessary to characterize the two periods by two different death rates,  $k_{\frac{1}{2}}$  the death rate of the first half second storage period and  $k_4$  the death rate of the subsequent 4 seconds storage period.

Two series of experiments were conducted at different combinations of temperature and R.H. In the first series the E. coli culture was prepared in distilled water suspension for aerosol generation, and in the other series in 0.85% saline water suspension. The  $k_{\frac{1}{2}}$  and  $k_4$  values found were recorded in Table 1.

In either case, the  $k_{\frac{1}{2}}$  and  $k_4$  values were found to decrease with increasing R.H. When the  $k$  values were plotted against a function of R.H., that is  $(100 - R.H.)$ , a family of straight lines were formed. Each straight



TABLE 1:  $k$  Values of *E. coli* Aerosols Sprayed from Distilled Water and Isotonic Saline Suspensions

Temp.	Distilled Water Suspension			0.85% Saline Suspension		
	R.H. %	$k_{\frac{1}{2}}$ sec <sup>-1</sup>	$k_4$ sec <sup>-1</sup>	R.H. %	$k_{\frac{1}{2}}$ sec <sup>-1</sup>	$k_4$ sec <sup>-1</sup>
20°C	20	2.68	0.085	20	3.40	0.130
	30	2.60	0.100	30	-----	-----
	40	2.46	0.075	40	3.10	0.093
	50	2.16	0.078	50	2.94	0.085
	60	1.55	0.066	60	2.82	0.071
	70	1.27	0.062	70	2.76	0.066
	80	1.04	0.062	80	2.80	0.058
30°C	20	3.41	0.130	20	4.06	0.140
	30	3.22	0.112	30	3.78	0.128
	40	2.82	0.102	40	3.44	0.121
	50	2.60	0.089	50	3.08	0.094
	60	2.52	0.083	60	2.94	0.086
	70	2.20	0.072	70	2.82	0.072
	80	2.00	0.060	80	2.86	0.070
40°C	20	4.75	0.165	20	4.56	0.146
	30	4.21	0.150	30	4.32	0.132
	40	3.66	0.130	40	3.90	0.143
	50	3.14	0.101	50	3.46	0.111
	60	2.88	0.092	60	3.20	0.100
	70	2.60	0.082	70	3.08	0.078
	80	2.01	0.060	80	2.94	0.081
50°C	20	5.87	0.185	20	5.10	0.162
	30	5.04	0.161	30	4.80	0.155
	40	4.45	0.142	40	4.42	0.147
	50	4.05	0.122	50	4.16	0.125
	60	3.30	0.116	60	3.82	0.122
	70	2.75	0.088	70	3.56	0.108
	80	-----	-----	80	-----	-----





line indicated that at a constant temperature, the death rate of airborne E. coli was in direct proportion to the decrease of R.H.

In the same way, when the k values for both cases were plotted in logarithmic scale against temperature in arithmetic scale, a family of straight lines was formed. This indicated that temperature affected the viability of E. coli aerosols logarithmically at constant R.H.

Figures 2 and 3 show the change of  $k_{\frac{1}{2}}$  values with R.H. and temperature respectively for E. coli sprayed with a distilled water suspension.

To compare the relationships between the death rates to R.H. and to temperatures with those of water evaporation to R.H. and to temperatures, equation (7) is used:

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

When water droplets are evaporated at a constant temperature and varying R.H., the previous equation becomes

$$\frac{dA}{dt} = \text{Constant} \cdot (f \cdot p_T - p_e) \quad (10)$$

where r, for very small particles, can be considered as a constant value at any instant, in the same way as Fuchs (19) treated very small particles in what he called the quasi-stationary state. This equation is in the form of a straight line function between the evaporation of water droplets, and the R.H. at constant temperature. The death of airborne E. coli and R.H. also bears the same relationship to one another as has been mentioned, (See Figure 2)

On the other hand, when R.H. is held constant while temperature is changing, equation (7) becomes

$$\frac{dA}{dt} = \text{Constant} \cdot \frac{\sqrt{\alpha}}{T} (f \cdot p_T - p_e) \quad (11)$$

Values of the function  $(f \cdot p_T - p_e) \sqrt{\alpha} / T$  were calculated with varying temperatures and the relationship of water evaporation with temperature was plotted



Figure 2  
 THE CHANGE OF RATE OF DEATH WITH RELATIVE HUMIDITY AT  
 CONSTANT TEMPERATURE (E. coli sprayed from distilled water suspension)

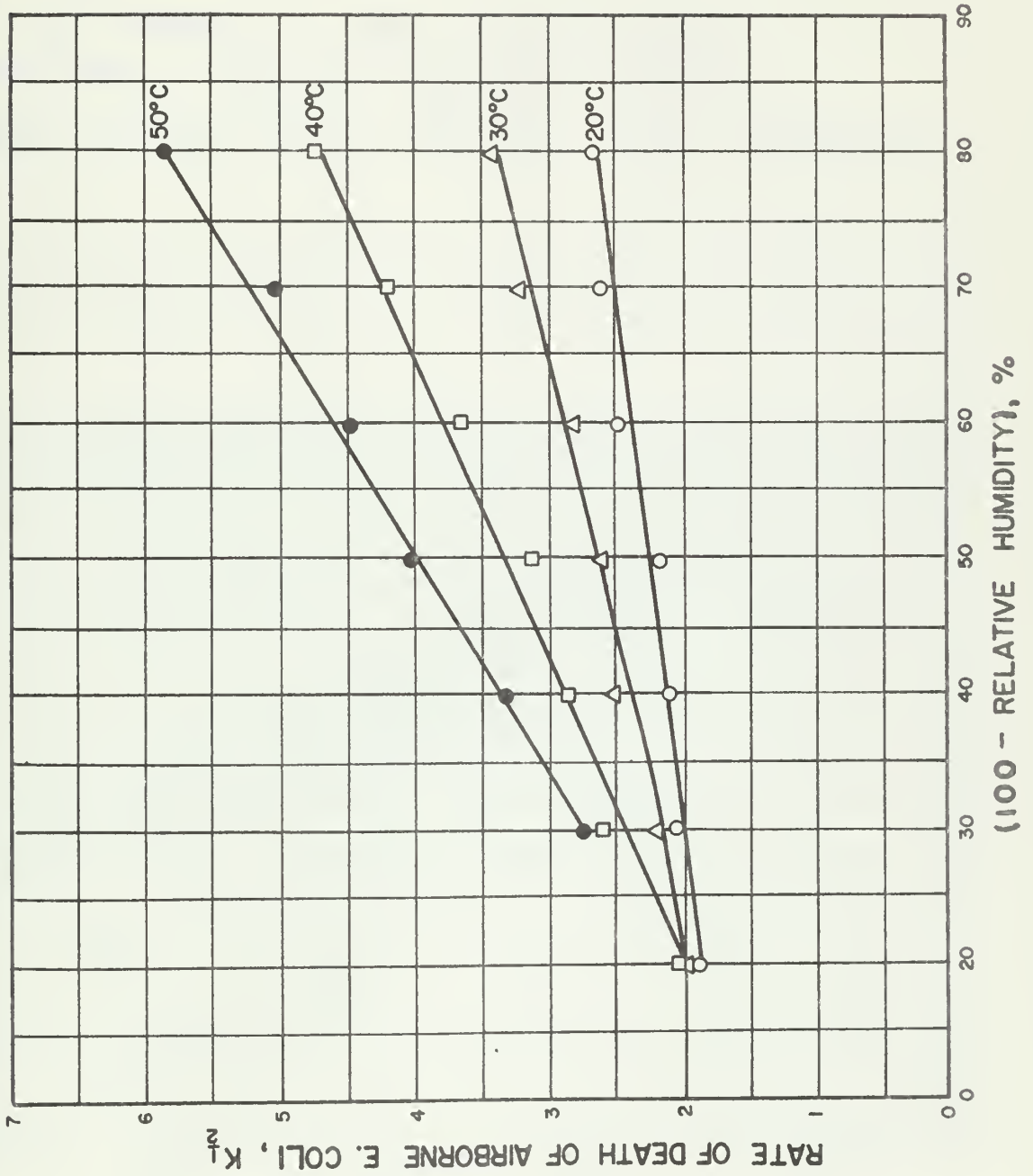
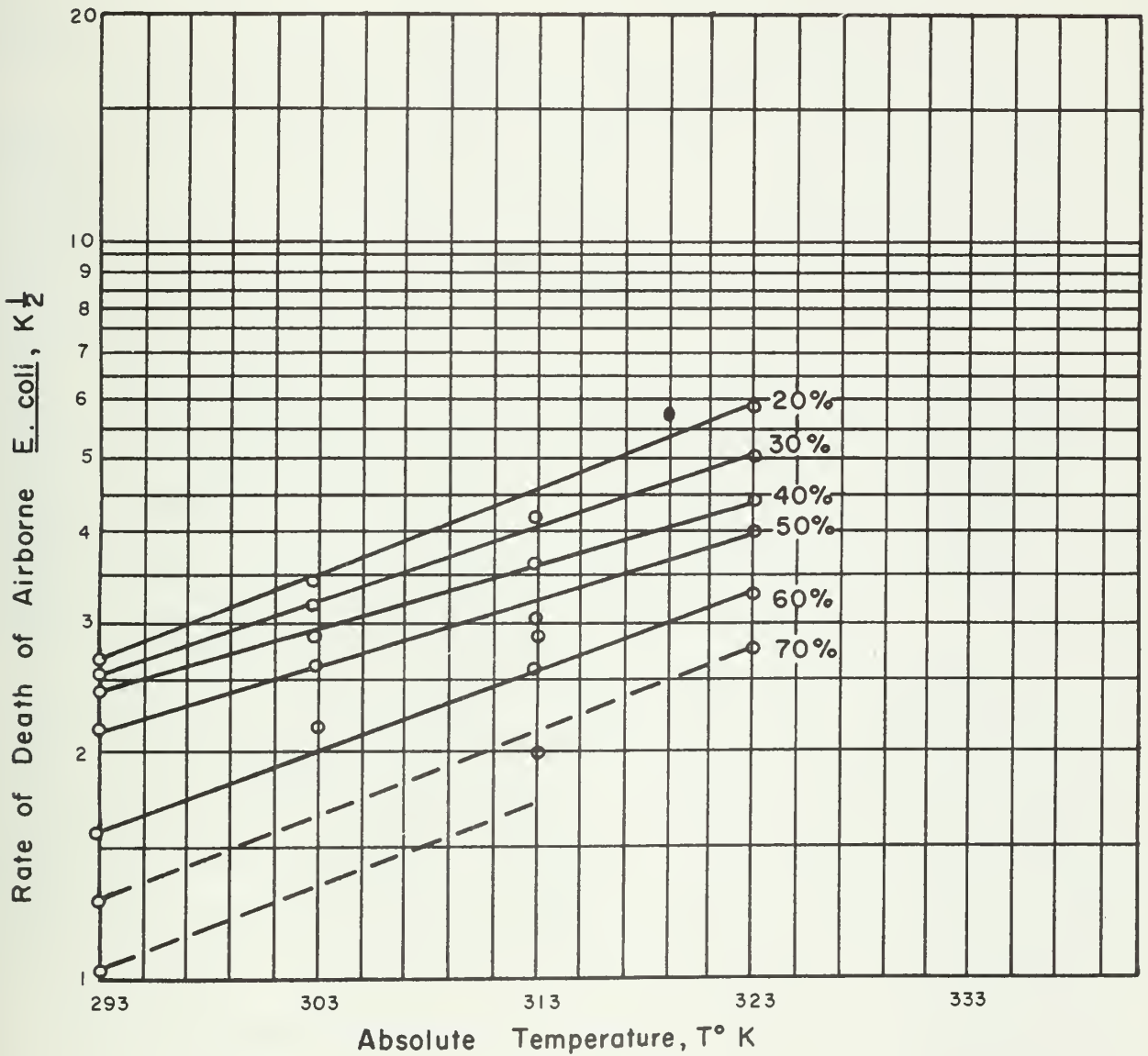




Figure 3

THE CHANGE OF RATE OF DEATH WITH  
TEMPERATURE AT CONSTANT RELATIVE HUMIDITY  
(E. COLI SPRAYED FROM DISTILLED WATER SUSPENSION)





in Figure 4 which shows that the evaporation of water droplets changes logarithmically with temperature as in the change of death rate of airborne E. coli shown in Figure 3.

In order to ascertain the combined effect of R.H. and temperature on water evaporation as compared to the death rate of airborne E. coli,  $k_{\frac{1}{2}}$  values were plotted against a function of water evaporation at varying R.H. and temperature as shown in Figure 5. An arbitrary unit was chosen for the function of water evaporation  $f (f.p_T - p_e) V / T$  in plotting Figure 5.

Straight lines were determined as being good fits for both cases. Since small water droplets evaporate instantaneously, it was felt that the function plotted in the figure truly represented water evaporation from bacterial aerosols and the instantaneous evaporation is the factor governing the viability of the airborne bacteria.

The presence of salt in the bacterial aerosols definitely affected the death rate by increasing the death of bacterial cells under the same condition of temperature and R.H. except in environments of high temperature and very low R.H. Under this condition, the airborne bacteria died more rapidly if they were sprayed from distilled water suspension.

In order to determine how a change in water evaporation would influence the rate of airborne bacteria, E. coli aerosols were sprayed from 5% saline and from 0.375% glycerol suspensions. Comparisons of the effects of the sources of aerosols are shown in Figure 6. It provides the evidence demonstrating the dependence of bacterial viability on water evaporation from cells. Because of the presence of salt in the bacterial aerosols, the bacteria died at a faster rate due to the dehydration effect of the salt and the death rate increased as the concentration of salt in the sprayed suspension increased. In the presence of some hygroscopic material such as glycerol, which impeded





Figure 4

## THE CHANGE OF RATE OF WATER EVAPORATION WITH TEMPERATURE

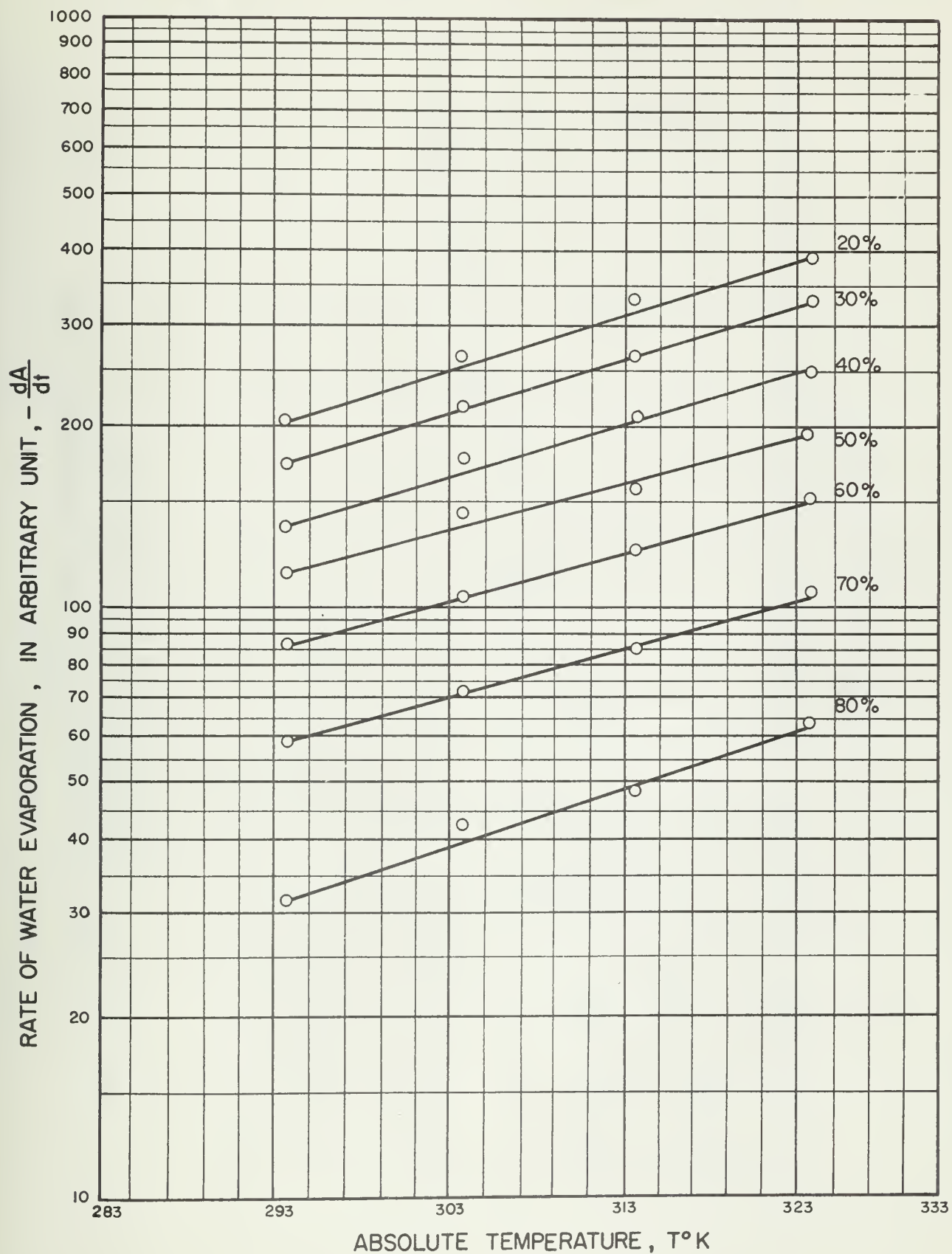




Figure 5  
 THE COMPARISON OF THE RELATIONSHIP OF THE  
 RATE OF DEATH OF AIRBORNE E. COLI FROM VARIOUS SOURCES TO THE  
 RATE OF WATER EVAPORATION

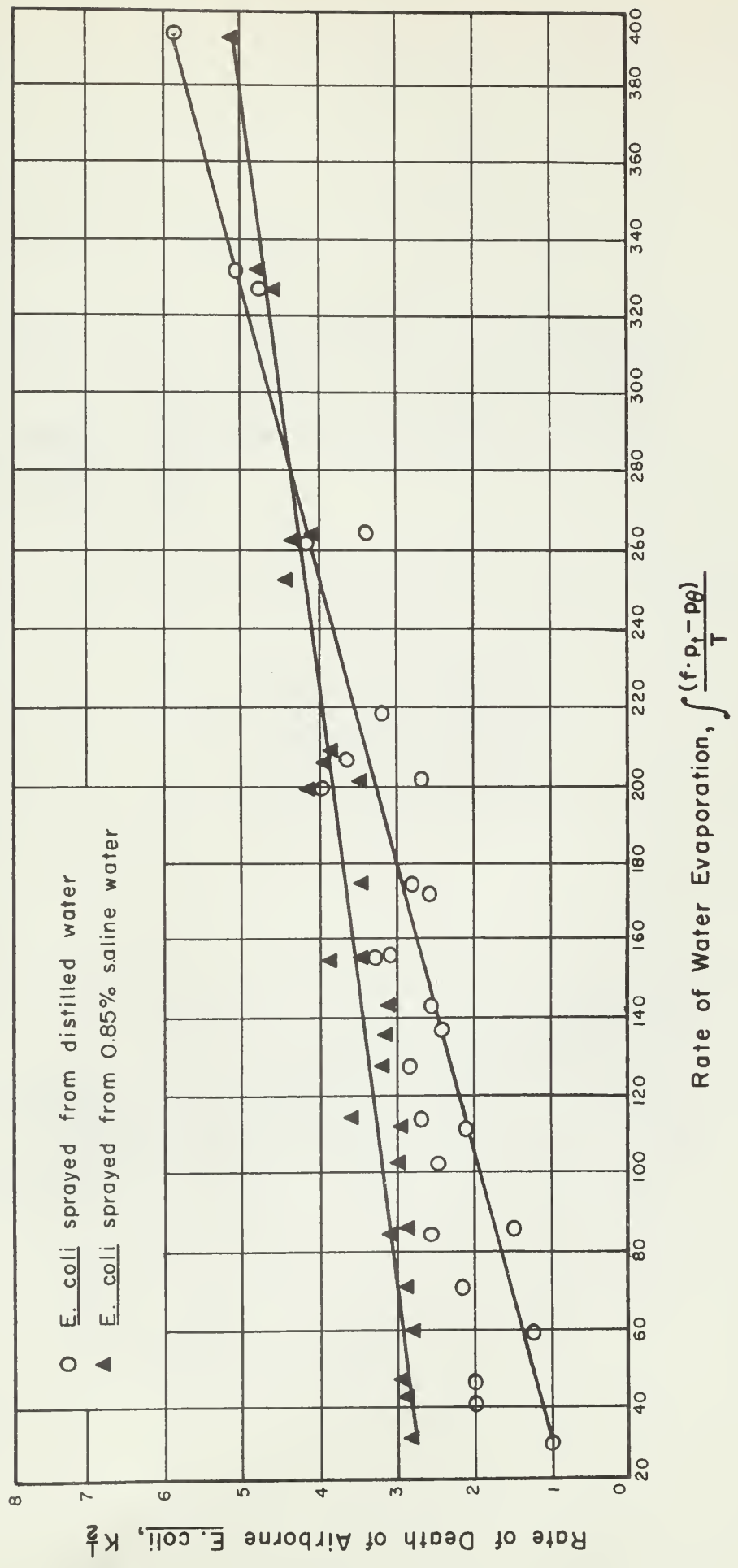
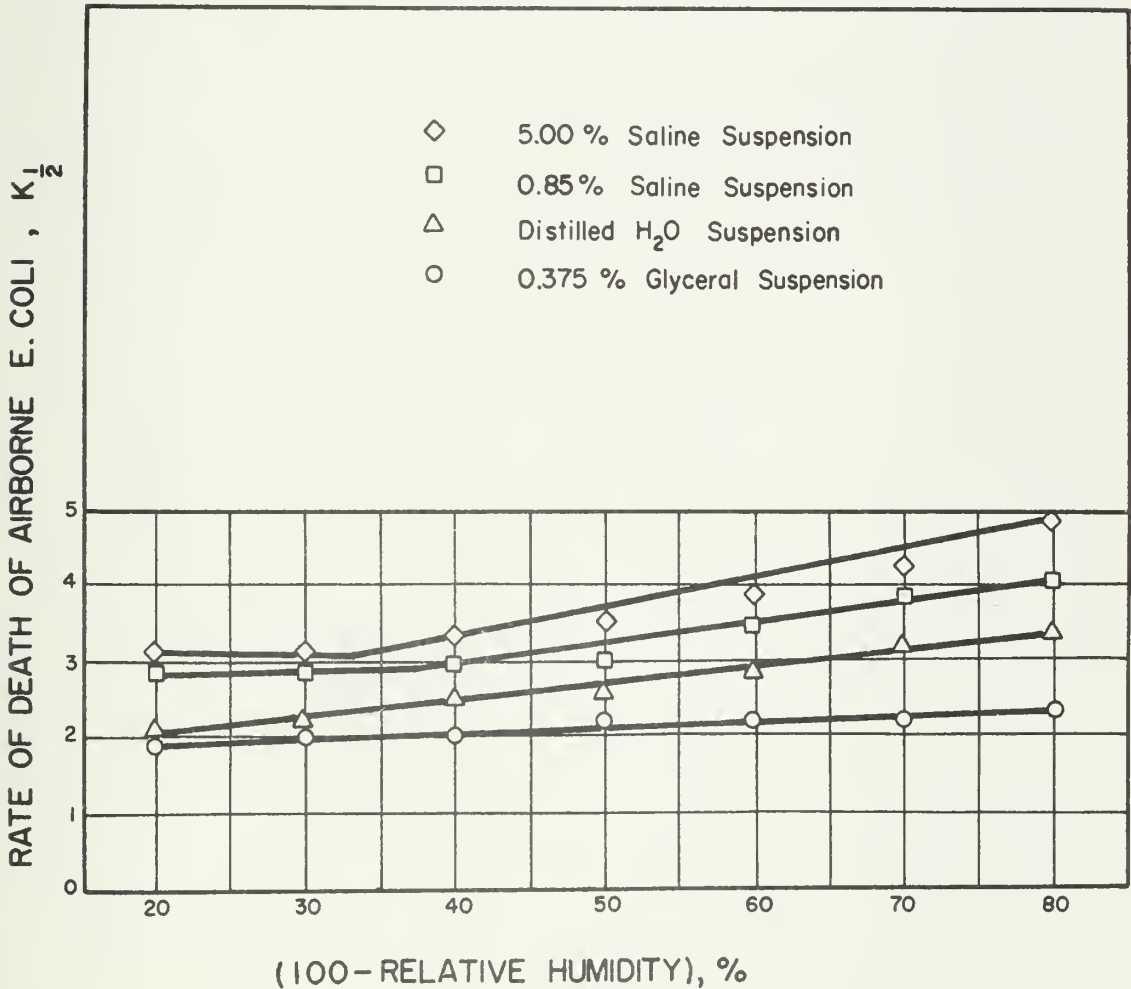




Figure 6

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.





the water evaporation, the death rate showed a considerable drop compared to bacterial aerosols sprayed from a distilled water suspension.

(b) The Effects of Flow Rate on the Viability of Airborne E. coli

A wind factor "f" was included in the equation of water droplet evaporation as suggested by Frössling to account for the change of evaporation rate influenced by the movement of air surrounding the water droplet:

$$-\frac{dA}{dt} = \frac{8 \pi D M}{R T d} (p_e - p_\infty) \cdot f \quad (12)$$

The wind factor  $f = (1 + K \sqrt{Re})$ , which exists only when the Reynold's number is large enough because K, a value obtained through experiments, depends on Re and K becomes extremely small if  $\sqrt{Re}$  is below unity <sup>(24)</sup>. The change of flow rate in the present study did not change the rate of death of airborne bacteria at all. This was expected since the Reynold's number in all the flows studied was less than unity with an average aerosol size of approximately 13  $\mu$ . The wind factor as a consequence was so small that it was neglected.





## VI. DISCUSSION

It has been found by many workers that viability of airborne microorganisms varied from genus to genus. The results of this study could therefore give information on the death rate of E. coli only. However, it is felt that there should be some common factors affecting the viability of airborne cells. Webb's data <sup>(18)</sup> showed that S. albus, B. subtilis, S. marcescens and E. coli varied very much in their death rates and yet all of them indicated an extremely high rate of death within the first second of storage and the subsequent death rate during the next 9 seconds was relatively slow. The same phenomenon was observed in the present study. This suggested one common mechanism which was responsible for the rapid decay of all these bacteria, regardless of the different structures of organisms.

The results also indicated that the highest mortality occurred at low R.H. and the rate of death decreased with increasing R.H. Paralleling this phenomenon is the evaporation of water droplets which takes place readily when they are exposed to an unsaturated air environment. A water droplet of 13  $\mu$  size at room temperature and R.H. will evaporate to 0.9  $\mu$  size, according to calculation, within four tenths of a second (See Equation 13). Suppose an aerosol of about 13  $\mu$  size is composed of a distilled water layer surrounding an average size of bacterial cell mass of 0.9  $\mu$ ; then it will take less than four tenths of a second to evaporate completely the water layer and leave the bacterial cells suspended in the air. Considering the 0.5 second storage time, the time period after the bacterial cells had been exposed to the air without the water layer was critical to the survivability of the airborne E. coli.

The rate of death for short storage time has been shown in this study to be directly proportional to the decrease of R.H. when temperature is constant.



The same relationship is found in the water evaporation rate. Furthermore, the rate of death of airborne cells for the short storage time at constant R.H. has been shown to be exponentially proportional to the increasing temperature. The rate of evaporation of water and the varying temperature bear the same relationship.

Webb (8) noticed a peculiar phenomenon of decreased death with increased temperature during the first second of aerosolization. This was however not observed in the present study as has been discussed. Webb explained his findings to be the result of a sudden chilling effect. It is obvious that the evaporation of the surface layer of aerosol water droplets would result in a sudden lowering of temperature at their surfaces. Therefore it was possible that some of the initial aerosol killed was produced by a large temperature drop. Hence, when the process of evaporation was carried out at a higher temperature the chilling effect was less. However, the temperature drop as calculated from Equation (6) did not lower the droplet temperature down to  $4^{\circ}\text{C}$  for all the temperature and R.H. levels investigated in the present study. Hence the sudden chilling effect on the bacterial aerosols can not account for the rapid initial killing.

The combined effect of temperature and R.H. on the viability of airborne bacteria is shown in Figure 5. The rate of death of airborne E. coli was directly proportional to the rate of water evaporation. It is evident that the rate of death of airborne bacteria differs from the rate of water evaporation. The difference lies in the fact that in these two different cases, the diffusing water molecules are crossing boundaries of different thickness for the completion of the evaporation process. In the evaporation of water droplets, a water molecule diffuses out of the spherical droplet into an imaginary boundary where the water molecule will have to travel a certain distance



before it collides with a gas molecule which completes the evaporation. A water molecule diffusing out from within a bacterial cell is further complicated by the presence of cellular membrane and the protein-water bond. The bond takes more energy to break it before the water molecule is free to travel and the cell membrane adds to the effective thickness of the imaginary boundary through which the water molecule has to travel for the completion of evaporation. It is therefore easily visualized that the rate of evaporation of water from within cells is much slower than that from water droplets alone.

It appeared from the plot in Figure 5 that as the  $f$  value became smaller, the spread of the individual points from the straight line became larger. This was thought to be due to the fact that at low temperature and high relative humidity, the rate of water evaporation was relatively so slow that there could be a significant lag period before the protein-bound-water started diffusing outward, consequently the relationship between  $k$  and the  $f$  values would not hold. In the case of aerosols from saline water, this possible lag period did not exist because of the immediate onset of dehydration of cellular water and therefore, the straight line was a better fit throughout all the  $f$  values in this case.

It was observed that in the case of *E. coli* aerosols from saline solution, the rate of change of  $k$  values was slower than in the case of distilled water. Another difference was that at low temperatures and high R.H., the  $k$  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 indicated in Figure 5.

The mechanism of the death of *E. coli* in both cases was identical. Regardless of the change of rate of death, the rate of water evaporation governed the rate of death. Nevertheless, additional factors beside temperature



and R.H. had their roles in affecting the evaporation of cellular water. 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 layer of a very high concentration of NaCl solution. In either cases, the evaporation process was retarded because the thickness of the barrier through which a diffusing water molecule had to travel was increased, plus the fact, that NaCl reduced water evaporation by lowering the vapor pressure in the solution.

b. "Dehydration" of protein molecules in the presence of a high salt content accelerated the water evaporation within the 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 osmotic pressure change can be tolerated by most bacteria to a certain extent. In spite of this fact, the osmotic effect was significant in the present study. As water evaporation neared completion outside the bacterial cells, the concentration of NaCl was so high that a very strong hypertonic solution existed which hardly any bacteria could with stand. Consequently cellular water was diffusing outward.

All these factors including the temperature and R.H. affected the water evaporation simultaneously and interacted with each other. For instance, higher osmotic pressure accelerated the "dehydration" process, but as more water was diffusing out, the concentration of sodium chloride was held 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 accompanied by a





new rate of death. This change of  $k$  values occurred at a slower rate in the case of aerosols saline suspension origin than that in the case of aerosols of distilled water origin, as shown in Figure 5.

The fact that at lower 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 the "dehydration" process. This process was much less affected by the factors of temperature and R.H. than by the other factors. Although the protein-bound-water evaporation as affected by low temperatures and high R.H. was slow, especially in the presence of NaCl crystals outside the cell membrane, the "dehydration" process at high NaCl concentrations readily took place and this was lethal to the bacterial cells. That was why the rate of death was higher. As the temperature increased and R.H. decreased, the limiting factor seemed to be the combined effect of temperature and R.H. on water evaporation. The consequence showed that in a high temperature and low R.H. region, the death rate was higher from distilled water origin. It might be that the effect of NaCl crystals in increasing the boundary layer between cells and their outer environment retarded water evaporation at a rate greater than the rate of increasing water evaporation by the effect of dehydration.

Glycerol in high concentrations is toxic to bacteria, but at a concentration of 0.375%, it causes no harmful effect on bacteria. It is a lipid, highly hygroscopic in nature. When bacterial aerosols were sprayed from glycerol suspension, water was not as readily evaporated as in aerosols sprayed from distilled water suspension. Two different characteristics were encountered in airborne bacteria sprayed from a glycerol suspension:

a. Due to its hygroscopic nature, the aerosols would tend to hold more moisture onto the cells, so it would take a longer time to evaporate them all.



In fact it was found in the present study that E. coli suspended in 0.375% glycerol, as examined by a Carl Zeiss phases microscope, showed an average size of somewhat between 1 and 2  $\mu$ , while the same culture suspended in distilled water showed an average size of less than 1  $\mu$ . This indicated that in glycerol suspension, E. coli had a higher water content.

b. The glycerol as a lipid material, would reduce the rate of water evaporation. It is known that in the presence of impurities in water particles, the rate of water evaporation is retarded. The same phenomenon occurred here. If we rearrange Equation (7) and integrate, we obtain

$$t = - \frac{R T d}{M \nu \alpha} \frac{r_o - r_t}{(f \cdot p_T - p_e)} \quad (13)$$

This is an equation for calculating the time required to evaporate a water droplet with radius  $r_o$  to a size of  $r_t$ . Anything which reduces the  $\alpha$  value would result in a longer time for the evaporation of the droplet. The glycerol here probably played the same role in reducing the  $\alpha$  value as the fatty alcohol did as reported by Eisner, Quince and Slack (29).

The phenomenon of abrupt change of the rate of death after 0.5 seconds was not very well understood. While a great portion of the population of bacteria died off in the first half second of storage, how the remaining portion maintained life is surprising. It is felt that this phenomenon has to do with the protein-water bond. Protein water can be linked to protein molecules through hydrogen bonds at various sites. Some of them link to the exterior of protein molecules whereas others could be trapped in the interior of the native protein. Some of them are possibly vital and some of them probably not. Some protein molecules might not even have water molecules in them. Chances were a great portion of the bacteria had lost the protein water which was vital and died off during that 0.5 seconds of storage and yet the remaining bacteria had lost only non-vital bonded water or had no bonded



water to lose at all. Those that managed to survive had only protein water which was strongly bonded and trapped in the interior of protein molecules. These water molecules were very difficult to evaporate not only because of the strong bond but because of their position inside the protein molecules which provided them with good protection against the process of evaporation. The rate of evaporation was therefore very slow and consequently the rate of death was relatively slow compared to that in the first 0.5 seconds of storage. In fact some of the bacteria could survive a long time as reported by others, probably because some protein-water was held inside indefinitely without evaporation.

Based on this explanation, the sudden change of the rate of death during the one half second of storage period suggested that within this period of time, both the free water outside the bacterial cells and the bonded water linked to the exterior of the protein molecules was mostly evaporated. Nevertheless the exact time when the bonded water molecules started diffusing out was not known. Since the lethal process actually started sometime within this one-half second storage time, the  $k_{\frac{1}{2}}$  values could be larger if they were calculated based on the exact lethal process time period instead of the one-half second storage time. For this reason, the  $k_{\frac{1}{2}}$  values reported in the present study did not represent the absolute death rate but instead gave the apparent death rate of this half-second storage time. It would seem that to compare the  $k$  values with the other research workers is impractical since no attempt has been made by any workers to find out the exact time when the rapid death rate did change to the relatively slow rate.

In order to find out this exact time, samples at one-tenth of a second intervals or even shorter should be taken. However, the design and precision of instrumentation would make this study very difficult at the present time.



The death rates were postulated to follow the first order reaction in short storage. However, it was felt that this was not necessarily true for all cases. Many workers have also postulated partial order reactions from first to fifth order reactions. The fact is that in the study of biological systems using death or certain inactivation as the end point for rate determination, the same reaction could go on in different parts of a protein molecule at different rates. The breakage of one protein-water bond may cause death or protein 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.

To compare the apparent death rate of the short storage, Webb's data <sup>(8)</sup> was used. 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% 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% R.H. was 1.14 and at 30°C and 50% R.H. was 1.35. The interpolated  $k_1$  at 25°C and 50% R.H. was 1.24 which was slightly smaller than that which Webb has found.

The study of velocity of the aerosol flow in the environment indicated no noticeable effect on the viability of bacterial aerosols within the range of velocities investigated. With wind factor  $f = (1 + K \sqrt{Re})$ , a particle of 13  $\mu$  gives a K value practically zero from Kinzer's experimental curve <sup>(24)</sup> and the wind factor becomes practically unity.





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## PUBLICATION, STAFF AND FOREIGN TRAVEL

## A. Publications

"Studies on Coagulation Employing Ammonium Chloride Aerosols"

J. of Air Pollution Control Association, 12, 266, (1962)

"The Effects of Humidity on the Coagulation Rate of Ammonium Chloride Aerosols" (in preparation)

"Short Storage Study on the Viability of Airborne Bacteria" (in preparation)

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Ichiya Hayakawa, Principal Investigator - Time as required,  
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<u>Degree</u>	<u>Institute Conferring</u>	<u>Field</u>	<u>Year</u>
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## Research Background:

<u>Institution</u>	<u>Nature</u>	<u>Year</u>
University of Illinois	Petrochemical Waste Treatment	1960-61
University of Illinois	Radon Gas Emanation from Soil Surface	1961-62





<u>Institution</u>	<u>Nature</u>	<u>Year</u>
University of Illinois	Coagulation of Aerosols	1962-present
University of Illinois	Factors Affecting the Viability of Air- borne Bacteria	1962-present

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Kanagawa Technical High School	Architecture	1953-62
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#### C. Foreign Travel

There has been no foreign travel associated with this research grant by August 30, 1963.

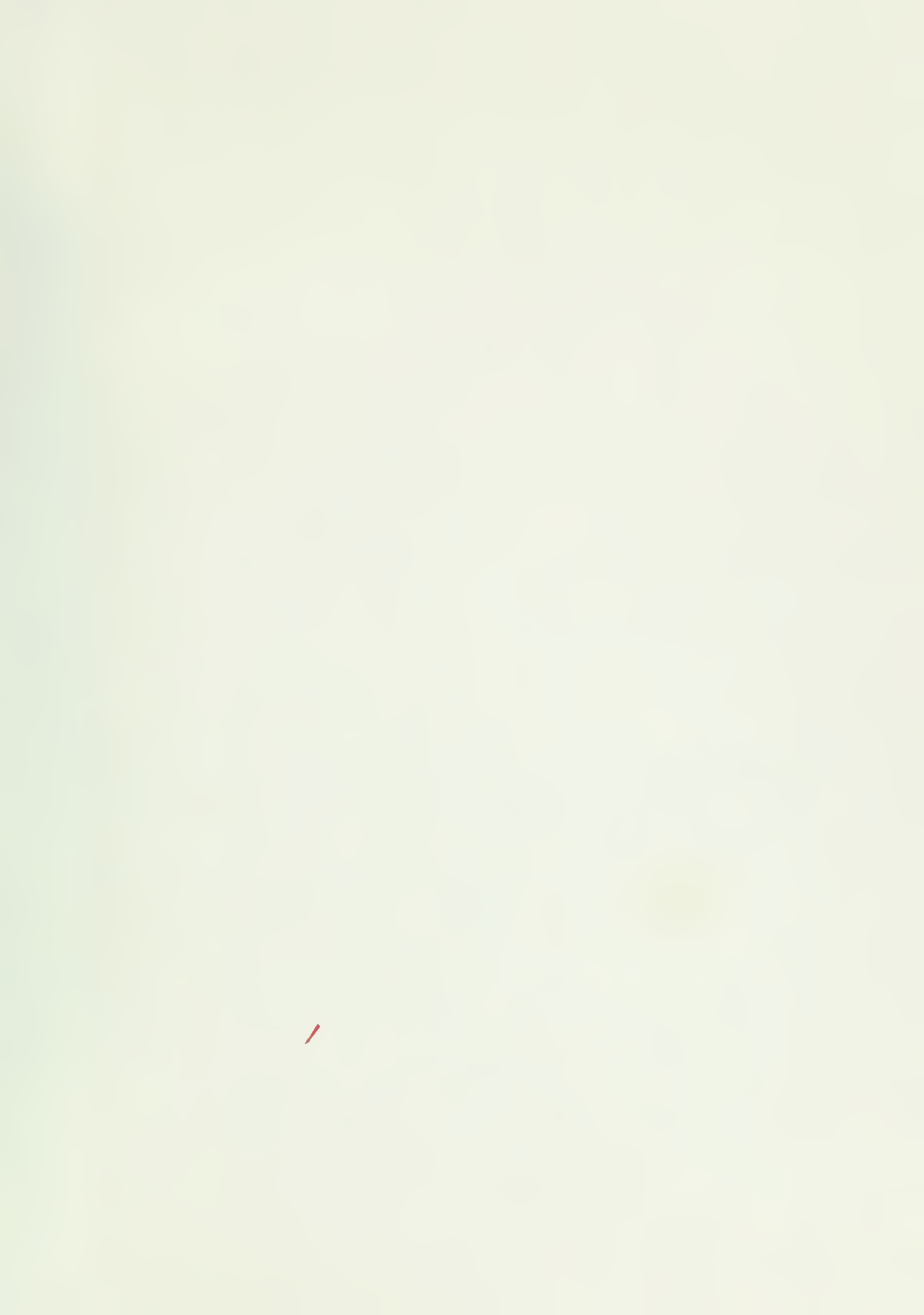












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