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PROGRESS REPORT

G-2771 (C-2)

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and

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Georgia Institute of Technology

Project B-100

AN INVESTIGATION OF THE EFFECTS OF MINUTE QUANTITIES OF CHEMICAL VAPORS ON BACTERIA AND SPORES

April 1, 1953 - February 1, 1954

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SUMMARY

Detailed studies leading to the development of a reproducible technique for the evaluation of aerial bactericides have been completed. The perfected technique has been applied to a study of air-borne Serratia marcescens under various temperature and humidity conditions. Chamber studies were made dynamically and statically to characterize both the physical and the biological responses of the air-borne bacteria. The results of these studies show a special lethal effect at intermediate humidities. The separation of the studies into dynamic and static runs has yielded more specific and detailed information than was previously available.

I. INTRODUCTION

The work described in this progress report has been carried out since March 31, 1953, the date the progress report was prepared for Grant-in-Aid E-141(C). In the earlier report the requirements of a method for studying the effects of chemical vapors on air-borne bacteria were discussed, and the accomplishments under this grant in developing techniques for such studies were described. It is the purpose of the present report to show more detailed evidence of the behavior of air-borne bacterial particles in the experimental chamber and to report our findings on the effect of temperature and humidity on the viability of air-borne S. marcescens. Reference will be made to the first progress report only when necessary to preserve continuity.

When this work was initially planned, it was anticipated that only a year would be required to perfect methods; however, it has taken nearly two years to accomplish this end. The primary reasons for conflicting reports in the literature on the survival of air-borne bacteria under various conditions were that the methods employed by different groups of workers were not yielding results comparable to those obtained by other groups employing different methods, and further, that insufficient attention had been directed to minor variables. It is believed that the techniques developed in our studies will be acceptable to most workers, and that both major and minor variables have been controlled.

This report presents under three headings the results accomplished during the past year: (1) the aerosol chamber and its accessories; (2) the effect of temperature and humidity on air-borne S. marcescens; (3) various miscellaneous work related to the study of air-borne bacteria. The supporting data are tabulated in Appendix I. Figures showing the interrelationship of such data are found in Appendix II.

II. THE AEROSOL CHAMBER AND ACCESSORY EQUIPMENT

In the previous progress report, types of chambers and equipment were discussed and reasons were presented for the decisions for finally selecting this particular chamber and equipment. No attempt will be made to review that work, but, for reasons of coherency, the actual physical equipment and procedures employed will be described in this report also.

A. Description

The aerosol work is carried out in a heavily insulated room 12 x 30 feet, 6 feet high. The room is divided into two sections, the larger serving as the workroom and the other as the control room for regulating the temperature and humidity. The controllable temperature range for this room is 0°-95° F, and the dew point of the air in the room can be controlled from 25°-95° F, resulting in a wide range of temperature and relative humidity. The degree of control, as shown by a recording dry-bulb and dew-point instrument, is within 1° F for both dry bulb and dew point.

Approximately 60 cfm of fresh, filtered air is continually pumped into the workroom through a stack having access to a point outside and above the Research building. This air is vented by the chamber pump during dynamic runs and through relief shutters when the chamber pump is not operating. This small amount of makeup air is sufficient, because operating personnel in the workroom is kept to a minimum and smoking is prohibited within the workroom.

The chamber is located in one corner of the workroom, about three feet from the side walls, and is on legs so that it is about 6 inches from the ceiling and 18 inches from the floor. Because the air in the room is recirculated at about 3,000 cfm, the narrow spacing between the top of the

chamber and the ceiling of the room is no deterrent to adequate heat transfer between the room air and the chamber. The chamber itself is a four-foot cube constructed of tempered Masonite and one side is removable. There are no internal projections in the chamber other than the air diffuser, and the inside surfaces were finished with several coats of a white alkyd resin enamel, each coat being hand rubbed to a mirror finish. There are 16 openings in the chamber walls: ten 3/4-inch holes for the taking of air samples, a 3/4-inch hole for connecting a manometer, a 3-inch hole in the center of the ceiling for the air inlet, five 4-inch holes equally spaced along a diagonal of the chamber floor, the center one serving as the air outlet, and the remainder are ports for settling samples.

The general layout of the chamber and atomizing equipment is shown in Figure 1. During the dynamic runs, the main air stream of the chamber (60 cfm) is drawn from the workroom through filter A and is metered by orifice meter C, passing through Anemostat D* and is discharged outside the workroom through filter F. The pressure withing the chamber is balanced to that of the surrounding room with the by-pass damper arrangement G, the pressure being read on manometer H.

Clean, regulated compressed air for the atomizer is metered at I and disperses the bacterial suspension in the atomizer J (DeVilbiss No. 40, run at 5.0 l/min.). The resultant aerosol suspension is forced into the 20-inch cubical prechamber K, where the large particles drop out. The air-borne bacteria are thoroughly mixed with the main air stream in the turbulence created at the orifice meter, and then they are uniformly distributed

* Trade mark of the Anemostat Corporation of America

throughout the main chamber by the Anemostat. Air samples are taken from any of the ports indicated in Figure 1. The settling-sample ports and containers are not shown in Figure 1.

During the static runs, the inlet and outlet to the chamber are closed off air-tight by means of slide valves located in these pipes just before they enter the chamber.

The Anemostat constitutes the only projection within the chamber itself. This diffuser is five inches in diameter and projects into the chamber about two inches.

Settling-sample containers are located beneath each of the four-inch holes in the bottom of the chamber. These containers have been machined out of one-inch thick die-stock and contain a sliding shutter which seals-off the hole in the chamber, a cavity for holding Petri dishes and a door on the bottom which can be closed air-tightly. In operation, all components are air-tight under at least one inch of water differential, so that when the sliding shutter is closed the chamber hole is sealed, a Petri dish can be inserted into the cavity of the holder, the bottom door of the holder closed, and, then, the Petri dish can be exposed to the chamber by opening the sliding shutter. Thus, it is possible to take settling samples as desired without ever disturbing the conditions within the chamber. Each settling-sample holder is equipped with a small air inlet and outlet, controlled by a three-way stopcock, in order to purge the cavity of the holder after the removal of exposed Petri dishes.

The inlet blower for the chamber draws air from the room or from a cubic-meter chamber which is employed for setting up concentrations of chemical

vapors to be studied. The exit blower for the chamber discharges outside of the workroom through a filter system.

The equipment for atomizing the bacterial aerosol is located near the inlet blower. In order to minimize changes in the bacterial culture during long runs, the atomizer and five-inch-diameter aspirator bottle used as a reservoir for the culture are immersed in a small water bath, the temperature of which is maintained at 45° F. Because the reservoir bottle has a much greater cross-sectional diameter than the atomizer, the level of the culture in the atomizer does not change appreciably for several hours, and it is possible to operate for as long as 10 hours by slightly changing the level of the reservoir bottle every two hours. The results concerning the numbers of bacteria in the chamber under varying conditions given in section III are proof of the value of this arrangement in standardizing the operating conditions.

For efficient operation, it is necessary to operate the atomizer continuously during the day, because at least one hour is required for equilibration within the prechamber. The main chamber equilibrates in about five minutes, and when the atomizer is operating constantly a new run can be started at any time.

In addition to the room in which the aerosol work is carried out, several other facilities are devoted to this work. The culture room and auxiliarly equipment are located on a balcony above the workroom so that sterile materials can be transferred directly back and forth. General laboratory and office space is located in another part of the building.

B. Operational Procedures

Operational procedures are best described by detailing the activities of a normal working day. First of all the workroom controls are regulated to establish the desired temperature and humidity. As these controls are only semi automatic until the set point is achieved, they require intermittent attention during the entire morning. The operator must adjust the temperature of the circulating water and the wet-bulb and dry-bulb thermostats in relation to the desired temperature and humidity, the ambient temperature in the building and the temperature and dew point of the outside air. The chamber blowers are turned on as soon as the control point is approached in order to equilibrate the chamber walls with the desired conditions. The air-purge system for the settling-sample holders is also turned on at this time.

During the morning the workroom floor, walls and other exposed surfaces are cleaned and mopped to prevent dust particles from being dispersed in the air during the runs. The inside of the chamber is not cleaned oftener than every few weeks.

The members of the group not in the workroom during the morning are engaged in preparing materials for the afternoon runs (agar plates cannot be poured long in advance because the surface will dry and form a skin), counting the plates of the previous day and checking and maintaining the various items of equipment necessary for the operation of the project.

The nutrient agar (Difco B-1) plates for the collection of settling samples during the static runs are prepared with approximately 80 ml of sterile agar. This volume of agar completely fills the previously sterilized Petri dish, so that when the dish is used for sampling, the surface of the agar is level with the chamber floor. The sterile agar used in the preparation

of these plates is cooled to as low a temperature as will be compatible with the sol state. This is necessary as too-warm agar results in excessive condensate between the Petri cover and the agar surface, causing spreading and coalescence of the bacterial colonies.

The nutrient gelatin (Difco B-11) plates for the collection of settling samples during the dynamic runs are prepared in a manner similar to that employed in the preparation of the agar plates. However, instead of completely filling the dishes, a volume of 15-20 ml is used. A greater volume would cause too great a dilution of the final sample, and because the sample is exposed for 20 minutes, the slight difference in height between the gelatin surface and the chamber floor is presumed to be inconsequential. After preparation, both gelatin and agar plates are stored in a refrigerator until used, none being stored for more than 48 hours.

All culture media--nutrient agar, nutrient gelatin and beef extract broth--are prepared in concentrations recommended by the Difco manufacturer on the labelled products.

The liquid medium used in the critical-orifice liquid-impinger samplers is a 0.2 per cent solution of Pharmagel A, buffered to a pH of 6.5-7.0 by the addition of 0.08 gram of anhydrous Na_2HPO_4 per gram of Pharmagel. To each impinger bottle (flat-sided quart milk bottles) is added 200 ml of this medium and 1 ml of olive oil to prevent excessive foaming. The entire sampling assembly is sterilized subsequent to assembling.

All glassware, culture media and collecting media are sterilized by autoclaving at 15-17 psi for 20 minutes.

While the materials for the runs are being prepared, atomization of the bacterial aerosol is started (usually at 10:00 A.M.). The atomizer reservior is charged with the contents of two flasks containing 40-hour-old cultures of S. marcescens (ATCC 274). Each flask contains 60 ml of beef-extract broth (Difco B-126) in which the culture was incubated at 30° C. At this time a 1.0-ml aliquot of the culture is withdrawn for dilution and plating-out for calculation of the culture count. The atomizer is placed in the empty cooling bath and is connected to the prechamber, the air supply and to the reservoir. The level of the culture in the atomizer is adjusted so that the tip of the atomizer jet is just clear of the liquid. The air pressure to the air supply for the atomizer is set at 30 psi, and the flow of air adjusted to 5.0 l/min. with a needle valve, the volume being indicated by a calibrated rotameter. The three-way stopcock at the exit of the prechamber is set to allow the flow from the prechamber to vent outside the workroom through a filter. The cooling bath is filled with water to a level slightly higher than the level of the culture in the atomizer and reservoir, and the circulating pump for the cooling bath is turned on. The cooling water is maintained at 45° F by storing a 30-gallon drum of water in an adjacent walk-in refrigerator maintained at this temperature. The circulating pump draws cold water from this drum, forces it into the bath for the atomizer and then returns it to the drum.

The atomizer is run continuously during the day. By starting it in the middle of the morning, ample time is allowed for the equilibration of the prechamber. For long runs the level of the culture in the atomizer is adjusted every few hours by changing the level of the reservoir.

For most runs the desired temperature and humidity have been achieved within the workroom by 11:00 A.M., and constant conditions are held for the rest of the day. To insure that the temperature and humidity of the chamber and chamber walls are completely equilibrated with the workroom air, the chamber blowers are kept running. All the previously described equipment is kept running continuously as set, until 1:00 P.M. During this time all items are checked intermittently to be certain of proper operation.

At 1:00 P.M. the aerosol stream from the prechamber is turned into the main air stream of the chamber and 15 minutes is allowed for the chamber to equilibrate. Three accurately calibrated critical-orifice liquid-impinger samplers (each of approximately a 1/min. capacity) are connected to air-sampling port at the side chamber, and four settling-sample holders are charged with Petri dishes containing nutrient gelatin. After the 15-minute equilibration time is completed, the impingers are connected to the vacuum source, and the shutters in the settling-sample holders are opened, and sampling is continued for exactly 20 minutes. At the end of this time, the vacuum is disconnected from the impingers, the impingers are removed and the air-sampling ports are plugged. Also, the shutters in the settling-sample holders are closed, the Petri dishes immediately are removed and covered,and the air purge is turned on for 30 seconds to purge the cavity of the sample holder of any residual aerosol. This is done with the bottom door of the holder removed.

The samples taken during the dynamic run are immediately removed to the culture room for plating. Other members of the group load the settling-sample holders with Petri dishes filled with agar and close off the chamber as quickly as possible following the removal of the samples and Petri dishes. Closing off the chamber is accomplished by shutting off the chamber blowers,

turning the three-way stopcock from the prechamber to vent the aerosol stream outside the workroom and closing the slide valves in the inlet and outlet pipes of the chamber--all as near simultaneously as possible. A stop watch is started and the static run has been initiated. Settling samples only are taken, as the air concentration under most of the conditions studied quickly drops so low that very large samples would be required. Settling samples are taken for periods of 60 seconds each, the first being started 60 seconds after the chamber has been closed off. Thus, number-one port is opened at 60 seconds, closed at 120 seconds, the sample is removed, immediately covered, inverted and placed in a can provided with a lid. The purge air is turned into the cavity of the sample holder, with the bottom door of the sample holder removed. At 180 seconds the shutter in number-two port is opened, the purge air turned off at number-one port and a fresh agar plate removed from a closed container and placed in number-one sample holder. At 240 seconds the shutter in number-two port is closed, and the entire procedure is repeated. This procedure is then continued, taking 60-second exposures at alternate minutes, moving sequentially from port number one to port number four and then back to number one until a minimum of ten samples has been taken. At all times fresh agar plates are held in closed containers until needed, and the exposed plates are immediately removed, covered, inverted and placed in closed containers.

At the completion of the static run the chamber is opened, the blowers turned on and the aerosol stream from the prechamber turned into the main air stream of the chamber. As before, 15-minutes equilibrium time is allowed and another dynamic run is started, followed immediately by a static run. This is repeated, giving three complete runs for the day.

The impinger and gelatin samples from each dynamic run are taken immediately to the culture room. Here three 1.0 ml aliquots are plated from the impinger fluid of each sampler. The gelatin is removed in toto from each dish to a sterile beaker containing warm, sterile water (50°), dissolved, transferred to a sterile 50.0 ml graduate, the volume made up to 50.0 ml, shaken and three 1.0-ml aliquots plated out. Nutrient agar (Difco B-1) poured at a temperature of 45° C is the nutrient medium. The plates so prepared are inverted as soon as they set up and placed in an incubator at 37° C for 48-56 hours prior to counting. If not counted within 56 hours the plates are stored in a refrigerator at 8° C until time is available for counting them.

The agar settling plates from the static runs are transferred to an incubator at 37° C for 24-36 hours prior to counting. If not counted at the end of 36 hours, they are removed to a refrigerator (8° C) and held until time is available for counting them.

C. Distribution of Bacterial Particles Within the Chamber

An intensive study of the distribution of the bacterial aerosol during dynamic runs was made shortly after the completion of the last progress report. This was before the final procedure given in section B was adopted, and the time intervals of taking samples were somewhat different. The results obtained at that time have been checked subsequently, but not in such detail, therefore these earlier data are shown to report on the distribution within the chamber. A total of more than 30 sets of replicate runs were made and analyzed. Only typical data are shown in Tables I and II. These data are for actual plate counts because calculating such counts into concentration per liter or settling per minute tends to disguise errors that are otherwise readily apparent. The information in Table I was selected to show the numerical deviations for counts which varied greatly.

The uniform distribution of the bacterial aerosol within the chamber is indicated by the relatively low standard deviations of the averages of the counts of viable organisms obtained from various sampling ports, as shown in Table I. These data also show the deviations obtained at different levels of numbers per actual plate count. The first column of counts in Table I was taken from runs where all 10 sampling ports were used; the second column was taken from runs where only three separated ports were used. A comparison between the deviations in the two columns shows that close agreement exists among all sampling positions. Corroborative evidence of this agreement was obtained from an analysis of the frequency with which any one port yielded counts higher or lower than any other port. At the 10 per cent level of confidence, the analysis indicated no significant difference between ports.

The data for settling samples shown in Table II are for five-minute exposure periods. These data show that there is no significant variation in the concentration of air-borne bacteria during dynamic runs. In general, the deviations in Table II are not significantly different from those in Table I for comparable averages.

Further checks on the distribution during dynamic runs repeatedly have confirmed these results. It has also been demonstrated that there is no significant numerical difference between air samples taken at the walls of the chamber (side and bottom) and those taken through tubes projecting various distances into the chamber.

In our work it has been assumed that if uniform distribution of the bacterial aerosol was achieved during the dynamic runs, then static runs would demonstrate the same uniformity of distribution. Various checks of

the variation between sampling ports has confirmed this, but the most convincing evidence is that obtained from extended static runs under conditions where a demonstrable number of the air-borne organisms were viable for at least an hour. The results of two such runs are shown in Figures 2 and 3. These two curves not only illustrate the linear trend of the data for numbers falling per minute, but also serve to illustrate two extremes in the quality of the data obtained in this work. In Figure 2 all points lie at or near the line drawn through them; in Figure 3 the points scatter more widely from the line. The latter situation occurred in only a few runs; most of the data obtained could be plotted in the manner shown in Figure 2.

One other point of interest is illustrated by these two plots. The run from which the data for Figure 3 were obtained was made several days prior to the run from which the data for Figure 2 were taken. It had been observed previously that as the numbers of bacterial particles falling per minute decreased below a count of about five per minute, the results obtained were always erratic. It was postulated that the area of the exposed Petri dish was small enough so that when the numbers falling per minute were less than five, the actual sampling became less and less accurate. It was theorized that if all four dishes were exposed simultaneously when these lower numbers existed, a more accurate sampling could be made. The last four points on Figure 2 were obtained in this manner (profiting from previous experience to use four plates on samples after 80 minutes), each point being the average of four plates. In Figure 3 all points are the counts for individual plates, and a wide scattering is manifested by the points obtained after 80 minutes.

To summarize: by comparing the values obtained at various single points with those obtained at any other, it has been shown that during dynamic runs a uniform distribution of the bacterial aerosol is achieved within the chamber.

The general, linear trend of the data from static runs shows that this uniformity of distribution is also characteristic of the static chamber, the only great scattering of data being observed when the numbers falling per minute become small and even this can be overcome by simultaneous exposure of several plates.

III. THE EFFECT OF TEMPERATURE AND HUMIDITY ON AIR-BORNE S. MARCESCENS

This work was undertaken because of the considerable importance attached to the effect of humidity on the viability of air-borne bacteria and because water vapor is a chemical belonging to one of the groups which were initially proposed for study. Furthermore, it has been reported that not only does humidity play an important role in determining the viability of air-borne bacteria, but it also has a profound effect on the bactericidal properties of chemical vapors, various humidities modifying them greatly.

A. Handling of the Data

All experiments were carried out in the chamber described in section II of this report, employing the procedures outlined there. Each run consisted of a dynamic phase during which time aerial and settling samples were taken, and a static phase immediately following the dynamic phase. During the static phase only settling samples were taken. The results obtained at various relative humidities at 60°-70° F are shown in Table III; for 80° F in Table IV; and for 90° F in Table V. In order to clarify certain expressions in these tables, it will be necessary to present a discussion of the methods employed in treating the original data. Under "Condition of Run," the relative humidity (R.H.) is expressed as per cent, and the dry bulb (D.B.) and dew point (D.P.) are expressed as degrees F. The culture count is that for broth culture which was atomized for the particular series of experiments, and the number of replicates refers to the number of complete runs that were made under the specified conditions with that particular culture.

During the dynamic section of the run, three critical-orifice liquid impingers, each accurately calibrated and of approximately 1 l/min. capacity, were operated simultaneously for twenty minutes. As the impinger bottles hold

200 ml of solution and three 1.0-ml aliquots are plated out, the average of the three plates so prepared divided by the exact capacity of the impinger and multiplied by 10 yields the concentration of viable airborne bacteria per liter of chamber air. Each value under the heading "Conc/L" in the following tables was determined by taking the average of the replicate runs for the particular humidity, temperature and culture. Also, during the dynamic section of the runs, four Petri dishes containing nutrient gelatin were exposed for twenty minutes then dissolved in warm sterile water to a volume of 50 ml and three 1.0-ml aliquots plated out. The average of all plates multiplied by 2.5 is then the number of viable bacterial particles settling per minute on a Petri dish during the dynamic phase. When more than one run was made for a particular condition with the same culture, the results of the settling studies were averaged. Because it was desired to compare the air samples with the settling samples, these results were translated into numbers settling per minute per 1,000 cm^2 by multiplying them by 15.4 (the ratio between the area of the dish and 1,000 cm^2). This particular expression was chosen because a liter volume occupies a 1-cm-thick layer over 1,000-cm^2 area, and therefore the ratio between the numbers settling per minute per 1,000 cm^2 and the concentration per liter is the rate of settling of the particles in cm/min. It should be emphasized that although both the air samples and the settling samples of the dynamic sections of the runs are based upon viable bacterial particles, both sampling procedures are carried out simultaneously upon the same aerosol, and the aerosol is constantly being renewed, therefore the number of viable particles presented to each sampling method should be identical, and the ratio between the settling samples and the air samples should indicate the physical characteristic of the particles just as if total particle counts had

been made. Reference is also made to section IV of this report where evidence is presented to show that over 90 per cent of the particles under study are composed of single bacterium. The somewhat less than 10 per cent of the particles which may be composed of two bacteria represent too small a proportion to cause significant variation from the case for single-bacterium particles.

For the reasons given above, the ratio between the number settling per minute per 1,000 cm^2 and the concentration per liter is shown in the following tables as "Rate of fall, cm/min." This information is then used to calculate the values shown under "Apparent Diameter, μ" by substitution in a simplified Stokes' equation:

$$v = 0.18\ d^2 \qquad \text{and}$$
$$d = \sqrt{(5.55)v} \tag{1}$$

where v is the rate of fall (cm/min.) of a spherical air-borne particle of unit density, and d is the diameter of the particle in microns (10^{-4} cm). The changes in the physical characteristics of the air over the range studied are insufficient to affect significantly the results obtained by such a simplified treatment. For lack of further information at the present time, the density of the particle is assumed to be unity.

The last column under "Dynamic Runs" in the tables is headed "k_f" and gives values which indicate the k due to natural fall-out of the particular particle in a four-foot chamber. It was desired to obtain this value in order to correct the data obtained from the static section of the run for the natural fall-out of particles, and thus to distinguish this effect from that due to the death of air-borne organisms. The general statement for such conditions is:

$$n = n_o e^{-Kt} \tag{2}$$

where n is the number remaining after the lapse of time, t, n_o is the original number and K is a constant. Rearranging equation 2 into logs base 10, gives:

$$K = \frac{(\log n_o - \log n)\ (2.3)}{t} \tag{3}$$

However, the die-away due to natural causes is ordinarily expressed as in equation 3, but with the value 2.3 (which is the factor for converting natural logs to logs base 10) deleted:

$$k = \frac{\log n_o - \log n}{t} \tag{4}$$

where k is the die-away constant employed in biological work. When t in equation 4 is set to the time required for the original number to fall by 90 per cent, or t_{90}, the following relation can be shown:

$$kt = 1 \text{ and } k = \frac{1}{t} . \tag{5}$$

However, k, in equations 4 and 5 differs numerically from K in equations 2 and 3 by a factor of 2.3. Another way of expressing equation 2 is:

$$n = n_o\ e^{-(\frac{v}{h})t} \tag{6}$$

where v is the rate of fall of the particles in cm/min., and h is the height of fall in cm (in this case, the height of the chamber). In this relationship between equations 2 and 6, $K = \frac{v}{h}$, but K is numerically 2.3 k, so

$$k_f = \frac{v}{(2.3)\ (h)} \tag{7}$$

where k_f (that is, the k caused by fall-out) is numerically in the same terms as the biological die-away constant. Equation 7 is used for the calculation of k_f from the data obtained during the dynamic runs. For the four-foot chamber, equation 7 is expressed:

$$k_f = (v)\ (0.00357) . \tag{8}$$

At the end of the dynamic section of the run, the chamber is immediately closed off and the static section is begun. Settling plates of agar are exposed for one-minute intervals under the extremely careful conditions described in section I. From 10-16 plates are exposed sequentially. The counts of these plates are then plotted on the log scale of semi-log paper against time on the arithmetic scale, and the best line drawn visually. Repeated checks of this procedure made by calculating the best line using the method of least squares has shown that the line drawn visually is verified by calculation except in those few cases where very scattered data have been obtained, and these runs have been rejected. Such few cases were found to be caused by improper operation of the shutter mechanisms in taking the settling samples. From the plotted line $\underline{k}_t$ is determined by use of equation (4). When more than one run is made for a particular condition with the same culture, the average $\underline{k}_t$ is determined by converting the individual values to the corresponding t_{90}, averaging and reconverting to $\underline{k}_t$. This is done because the $\underline{k}$ values are logs and cannot be averaged directly.

The second column under "Static Runs" in the tables is headed "Net $\underline{k}$ Due to Death," and the values here are obtained by subtracting $\underline{k}_f$ from $\underline{k}_t$, thereby cancelling out the die-away effect due to the natural settling of the particles and leaving only the die-away due to the death of the organisms. It should be noted that the values for $\underline{k}_f$ are so much smaller than the values for $\underline{k}_t$ that this correction has little effect on the final $\underline{k}$. However, under more favorable conditions where the death rate is not so rapid, such a correction would become more necessary.

B. Results of the Dynamic Runs

As indicated in A, the results of the dynamic runs were intended to be used primarily to characterize the physical attributes of the airborne bacterial particles. However, a close study of the results indicated that some conclusions of biological importance could also be obtained. The basic information concerning these runs is shown in Tables III-V. The values for air concentration and for settling were first translated into terms of a common culture concentration. The conditions for atomizing the culture, described in section II of this report, are such that the number of bacterial particles in the chamber should be the same from run to run, if the number of bacteria per millilter of atomizing culture remained constant. Reference to Tables III-V shows that the culture count varied from 45-75 x 10^7/ml, in Tables VI-XI, the values for air concentration and for settling have been translated into values in terms of a common culture of 100 x 10^7/ml, thus allowing the examination of such other variables as might affect the numbers of bacterial particles in the chamber. The information concerning numbers in the air for the runs at the various temperatures and humidities is shown in Figure 4. In this chart numbers of bacteria per liter of chamber air are plotted against relative humidity for 65°F, 80°F and 90° F.

In examining the curves shown in Figure 4, two observations can be made; temperature has a depressing effect on the numbers of bacteria, and the three curves appear to have a common shape. The effect of temperature might well have been predicted from the cultural response of S. marcescens, it being a mesophilic organism. It would therefore follow that temperatures above optimum would have an adverse effect on viability,

even in the air. In this particular instance, the lethal effect of temperature is undoubtedly manifested in the prechamber rather than in the chamber for the following reasons: the prechamber temperature is determined by the ambient temperature of the workroom, rather than by the atomized culture stream; the equilibrium time of the prechamber is much longer than that of the chamber (about one hour as against approximately five minutes), therefore the lethal effect of temperature can be exerted before the bacteria leave the prechamber. We are, therefore, dealing with a factor which affects the bacteria before they enter the chamber.

The peculiar shape of the curves in Figure 4, demonstrating an inflexion at some intermediate relative humidity, is not so readily explained. It is not possible to ascribe this phenomenon to changes taking place in the prechamber because the atmosphere within the prechamber always stays at or near saturation with respect to water vapor. Therefore, it must be assumed that the causation occurs between the prechamber and the main chamber inlet. The chamber air stream is moving at 60 cfm through about 10 feet of two-inch pipe, therefore less than 20 seconds lapses between these two points. If bacteria are dying during this time, the effect must be virtually instantaneous. If this is the case, it is possible that the rate of drying of the bacterial particles is involved, as has been suggested by other workers.* Thus at the lower relative humidities desiccation is so rapid that a stable, viable particle results; as the relative humidity increases, the drying is somewhat slower and a greater number of bacteria die during the process. The exact location of the most unfavorable relative humidity

* Dunklin, E. W., and Puck, T. T., J. Exp. Med. 87, 87 (1947).

appears to vary with the temperature. At relative humidities above this unfavorable condition the amount of drying is less, and as the relative humidity increases the bacteria exist in a more favorable environment. Although this explanation appears to describe the situation, it is possible that other factors may be responsible. Further consideration of this phenomenon would seem to be warranted.

The information concerning the number of bacterial particles settling in the chamber for the runs at various temperatures and humidities is shown in Figure 5. In this chart numbers of bacteria settling per minute per 1,000 cm^2 are plotted against relative humidity at 65° F, and 80° F and 90° F. Comparison of the curves in Figure 5 with those in Figure 4 shows that the same factors appear to be operating, except that in Figure 5 an extra inflexion is manifested at the lower humidities at 80° F and at the higher humidities for 65° F. If the factors responsible for the changes in numbers with varying temperature and humidity were exerting their influences before the bacteria enter the chamber, the curves in Figures 4 and 5 should be essentially identical. The general similarity of the curves supports this contention, but the two points of difference indicate the possibility a change taking place within the chamber itself. It is possible that this may be caused by the different methods of sampling employed -- the air samples being taken up in a liquid, and the settling samples falling on a solid surface. However, the difference is not in the expected direction in that the settling samples are <u>greater</u> at the points in question than might be expected from the aerial samples. No immediate explanation has been offered to account for this difference.

In order to determine the physical characteristics of the bacterial particles within the chamber, the rate of settling (shown in Tables III-V) was determined as the ratio of the settling samples to the air samples. The ratio, as determined in this case, should be the rate of fall of the particle, in cm/min, assuming that the methods of air and settling sampling are comparable,and that they are sampling the same population. The fact that the curves in Figures 4 and 5 are generally similar verifies this assumption, but the two points of difference between these curves tend to militate against it. In Figure 6 these ratios are plotted against relative humidity for the various temperatures. The suspicion that some unfavorable factor within the chamber is operative is partially confirmed by the observation that the three curves in Figure 6 do not seem to arise from the same causes, i.e., the shapes of the curves for 80° F and 90° F are somewhat similar, but different from that of the curve for 65° F. However, the fact that there is much less variation due to temperature tends to indicate that the major changes in numbers take place before the bacteria enter the chamber. The only relation between rate of fall and relative humidity that might be expected is that suggested by other workers who have assumed that particle size (and rate of fall) increases with increasing relative humidity. However, if such is the case, one would not expect to find a very marked increase under the experimental conditions for the work reported herein, because the majority of the particles are single bacterium particles and have only a very small amount of foreign matter associated with them. It could not be presumed that a living cell would vary in size with varying relative humidity in the same manner as would inert material.

To summarize the results of the dynamic runs: in general the results indicate that we are justified in taking the ratio between the settling samples and the air samples as a function of the rate of fall of the particles, but results are not entirely in accordance with this premise, some possible difference in sampling response being indicated at certain humidities. In general, the total numbers of viable organisms entering the chamber is diminished with increasing temperature in the actual run, death apparently taking place in the prechamber. Some further lethal factor, possible rate of drying, related to relative humidity, seems to be operative during the time the bacteria pass from the prechamber to the main chamber.

C. Results of Static Runs

The results of counts on sequentially exposed plates during the static runs were plotted, and the $\underline{k}_t$ values were determined from the resultant line, as described in A. The effect of natural fall-out of particles was removed by subtracting the $\underline{k}_f$ (determined from dynamic runs) from the $\underline{k}_t$, thus giving the net $\underline{k}$ due to death of the bacteria alone. These values are shown in the last columns of Tables I-III. In Tables XII-XIV these numbers are shown as $\underline{t}_{90}$ value which is the reciprocal of $\underline{k}$. For the purpose of demonstrating viability $\underline{t}_{90}$ values are more readily handled because the $\underline{t}_{90}$ values increase numerically with increasing survival.

In Figure 7 the results of the static runs, showing the effect of temperature and humidity on the viability of air-borne S. marcescens, are plotted as $\underline{t}_{90}$ values against relative humidity for 65° F, 80° F and 90° F. Examination of the curves in Figure 7 shows that the generally

unfavorable effect of increasing temperature demonstrated from the results of the dynamic runs (Figure 4) is also manifested in the static runs. However, at the lower humidities there appears to be little difference in effect between 65° F and 80° F, indicating a sharp change in the temperature response above 80° F. A more detailed study would be required to determine whether this is indeed the case, or whether there is a more gradual response to increasing temperature as indicated by the curves in Figure 4.

The effect of humidity on the viability of the air-borne S. marcescens, as demonstrated in Figure 7, is quite striking. Except for the curve for 65° F, the appearance of these curves is similar to those published by earlier workers,* i.e., a special lethal effect seems to exist at or near 50 per cent relative humidity. The earlier workers have suggested that this might be caused by unfavorable concentrations of salt (from the atomizing medium), the lower humidities protecting because of the rapidity of drying. These workers reported that dialyzing the atomizing medium before their experiments or deleting NaCl from the suspending medium resulted in a diminution of this special lethal effect. However, in the work reported herein, the particles under study are predominantly single bacterium particles and are atomized by an efficient atomizer which probably puts them into the air as droplets not much greater in size than the bacterium itself. Thus, there is hardly more than a film of adherent medium surrounding the bacterium. Furthermore, the concentration of salt in the dispersing medium is low to begin with, being about 0.015 per cent. The total solids content of the dispersing medium is only 0.30 per cent.

*Dunklin, E. W. and Puck, T. T. J. Exp. Med. 87, 87 (1947)
Lester, W., J. Exp. Med., 88, 361(1948)

Comparison of the effect of the lower humidities on survival as shown in Figure 7 with those effects shown in Figure 4 and referred in B previously mentioned definitely indicates that the rate of desiccation may be very important in determining viability. The results of the dynamic runs show that for each temperature there is some humidity that shows a special lethal effect; at 65° F it is about 50 per cent at 80° F about 40 per cent and at 90° F about 25 per cent. The analysis of the conditions under which this occurs having indicated that this effect of humidity probably manifests itself in a very short interval of time, and having assumed that rate of drying would explain the phenomenon, it would be predicted that the results from the static runs should show that these humidities at the particular temperatures are much less lethal than they are in the dynamic runs. This is because these bacteria having survived the intermediate rates of drying should be able to continue to live under those particular conditions, i.e., having survived the instantaneous effect of an intermediate rate of drying, the special lethal effect of the particular relative humidity should no longer be manifested. Comparison of the curves in Figures 4 and 7 yields some verification of this prediction. Those relative humidities which show a special lethal effect in the dynamic studies show an effect in the static runs not greatly different from the immediately higher or lower humidities. This shift is especially marked at 65° F, where there seems to be a reversal of the special lethal effect at 50 per cent relative humidity. The differences are much more marked than is immediately evident from a comparison of Figures 4 and 7, because the values in Figure 4 are for numbers surviving and those in Figure 7 are for the time required for 90 per cent of the bacteria to die.

To summarize the results of the static runs: at 65° F, 80° F and 90° F, for air-borne S. marcescens, the higher and lower relative humidites are more favorable for survival than are the intermediate humidities, although at 65° F there is an indication of a reversal of this trend. In general, increasing temperature between 65° F and 90° F has an increasingly unfavorable effect upon survival, the effect being more pronounced at the intermediate and higher humidities than at the lower humidities.

D. Discussion

An attempt has been made in the body of this section to discuss the salient points raised by the results obtained in our study of the effects of temperature and humidity on the survival of air-borne S. marcescens. The nonlinear trends demonstrated by the results have made it impossible to initiate any constructive interpretation until this work was completed, within a week of the writing of this report. No attempt was made to synthesize any generalizations; however, the nature of the information so far obtained is excellent proof of the usefulness of the methods developed in our work. Furthermore, extending this phase of the work to include several more temperatures should yield information which would permit the elucidation of a general statement of the exact effect of temperature and of relative humidity in determining the viability of air-borne organisms. Obviously, to verify such generalizations, it would be necessary to repeat such studies on other test organisms.

IV. STUDIES ON OPERATIONAL VARIABLES

A. Action of the Impingers

When it was realized that the apparent diameter of the bacterial particles as estimated from the comparison of aerial and settling samples was generally larger than that obtained by other methods, a thorough study of the two methods of sampling was made. For erroneously high values of apparent diameter, the results from the settling plates would have to be erroneously high, or those from the impingers erroneously low. No consistent error was found which would account for the numbers on the settling plates being high (discussed in the next subheading) so it was assumed that the error, if any, was caused by some fault of the impingers

It is virtually impossible to check the efficiency of a critical-orifice impinger directly; because of possible adverse effect of the low pressure existing on the exit side of the impinger, the fate of any bacteria passing through the impinger is open to question. It has therefore been necessary to estimate the efficiency of these impingers (for the particle sizes under study) by indirect methods. However, the fact that total numbers and internal ratios could be reproduced time and again day after day during the study of the effect of temperature and humidity shows that regardless of the absolute efficiency of the impingers, the results obtained from them with particles of the size under study were most reproducible. For studies such as the present, absolute efficiency is to be desired, but reproducibility is a necessity.

In order to determine whether or not bacteria are lost or die in the impinger solution during sampling, several samplers were charged with sterile impinger fluid to which were added known numbers of bacteria.

These impingers were then operated at critical flow for a total of one hour, the inlet air being drawn through a filter to prevent excessive control contamination from the air. At intervals of 15 minutes, samples of the impinger fluid were removed from the samplers for the determination of the bacterial counts. No significant difference was observed in the counts before, during and after the completion of the test period, indicating that bacteria are neither lost nor die in the impinger fluid during sampling.

The work in other laboratories* being carried out concurrently with the work reported herein indicated that critical-orifice impingers have a very high efficiency for the collection of particles of the size under study. We have, therefore, extended our own studies in an attempt to verify this result. The results of several-hundred impinger samples merely verified the reproducibility of the method of sampling. High-speed moving pictures (4,000 frames per second) and microflash stills (4 microseconds) were made of the tip of an operating impinger in order to obtain information as to the mechanism of the sampling action. It was hoped that having determined this mechanism, it might be possible to follow relatively large particles (10-15 microns) through the impinger and estimate the probability of smaller particles acting in the same manner. Unfortunately, no single interpretation of the mechanism of the sampling action has been agreed upon following a study of the film. In general, it appears that a steady jet of air emerges from the critical orifice, that this jet travels several millimeters from the tip of the orifice and then explodes into bubbles, the bubbles being formed at about 150 per minute per liter of air sampled. Such a picture is not consistent with a simple

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*U.S.P.H.S., C.D.C., Savannah, Ga.

impaction mechanism, but no acceptable explanation for the mechanism has been offered.

It is our present conclusion that the critical-orifice liquid impinger is an extremely reproducible method of sampling bacterial aerosols; we have no direct evidence as to the actual mechanism of action nor the absolute efficiency of these samplers. The reproducibility, ease of use and manufacture of these samplers commend them to the studies at hand in spite of our lack of direct evidence as to their efficiency.

B. Errors in Taking Settling Samples

During sampling of settling particles by means of concurrently exposed agar plates, a progressive discrepancy was noted in the number of settled bacteria, the number increasing with increasing time lapse between closure of the shutter and removal of the plate from the sampling container. An investigation of this increase in numbers (as high as 20 per cent) showed that it could be eliminated by removing the plate immediately after the completion of the exposure. This seemed to indicate that the error was caused by the inclusion of particles from the chamber in the cavity of the settling-sample holder, and that these particles then settled on the plate causing a larger number of colonies than would be the case when the plate was removed immediately. This surmise was supported by the fact that the increase in numbers was always a function of the numbers in the air and was not a random occurrence.

In order to obviate this error an air inlet and outlet system (described in section I) was made for the cavity of the sample holders. The air exchange rate through this system is sufficiently great to prevent any accumulation of particles in the cavity of the sample holders, and through its use it is

possible to leave plates in the sample holder without incurring an error due to extraneous particles. Even when the plate is removed immediately, the air-purge system is turned on to sweep out the cavity and to make doubly certain that errors of this sort are not encountered.

C. Particle Size and Composition

The function of the prechamber is to prevent particles having a diameter greater than 3-4 microns from entering the chamber. This range of size was selected for cut-off because it was estimated that particles containing more than two bacteria would be equivalent to spheres of that diameter, and setting up an aerosol composed primarily of single bacterium particles was desired. During the earlier runs, before it was possible to predict the numbers which might be expected to settle during a specified time interval, it was customary to include both gelatin and agar plates for taking settling samples. Because the gelatin samples could be dissolved, diluted and aliquots of the solution plated out for counts, it was thus possible to obtain accurate counts for numbers that would have overcrowded the agar plates, which are counted directly. As the test organism, S. marcescens, does not form chains or otherwise clump, it would be expected that any particles containing more than a single bacterium would be broken up and seperated into individual bacterium during the process of dissolving and diluting the gelatin samples. Since the bacterial particles settle directly on the solid agar and are allowed to form colonies just as they fall, the counts on the agar plates would thus be counts for the total number of particles; whereas the counts from the gelatin plates would be expected to be counts for the total number of bacteria.

The results of 10 consecutive runs are shown in Table XV. It will be noted that the second column lists the direct counts for the agar plates, and the third column lists a probable number for this value, corrected for overlapping. A consideration of the situation existing during the taking of settling samples shows that just as soon as one particle has fallen, there is some probability that the next particle will fall in the same spot. Now if two particles fall on the same spot, they will form a single colony and be counted as one particle. Even if they fall no closer than 0.5 mm to each other, there is an excellent chance that the resultant growth will be counted as a single colony. As the number of particles that has fallen on the plate increases, the probability of overlapping increases rapidly. The mathematics of this situation has been given considerable attention, and we have used the equations from a recent article* to estimate the probable number represented by a particular count. It should be pointed out that the variation between these two numbers becomes great once the total number of particles falling on a Petri dish has become as large as 100.

To ascertain the relation between the counts from the agar and gelatin plates the fourth column in Table XV was inserted. It will be noted that there is no considerable variation in the values listed in this column, and the average is 93. From this it was estimated that not more than 10 per cent of the bacterial particles contained more than a single bacterium.

To verify the conclusion drawn from the data shown in Table XV that the aerosol particles in the chamber were almost all single-bacterium-containing particles, electron-microscope screens were placed on the floor and left

* Mack, C., Biometricka 40, 220-22 (1953).

during a total of 48 hours of dynamic runs. This long period of exposure was necessary because of the small size of the squares of the electron-microscope screens (50 micron square). Under the electron microscope it was found that no particles having as many as three bacteria were present on these screens; 5.5 per cent of the observed particles definitely contained two bacteria per particle, 5.5 per cent of the particles were made up of single bacterium in the process of dividing or possibly two bacteria joined end-to-end and 89 per cent of the observed particles contained a single bacterium per particle.

In examining the screens under the electron microscope, it was noted that the bacteria were rod-shaped, approximately 1.5 x 3.0 microns, although some were as large as 1.5 x 5.0 microns. A capsule 1.5 x 3.0 microns would have a mass equivalent to a spherical particle of 2.2 microns diameter. Such a value is slightly higher than the average obtained from cascade-impactor studies; these indicated a probable diameter of 2.0 microns. Both of these values are much lower than those obtained by comparing aerial concentration to settling samples. In this case the apparent size seemed to vary with relative humidity, going from 2.5 microns to as high as 4.5 microns in diameter. (See section III.) No valid reason for the discrepancies in particle size as determined by these methods has been offered. It is possible that all are essentially correct, because the impactor and electron-microscope studies were made before it was known that humidity might affect the particle size significantly.

D. Direct Counting of Bacterial Particles

Some method for routinely estimating the actual numbers of bacterial particles in the chamber would be desirable, as distinguished from the estimation

of viable bacteria. Such a method which will be useful, should be one requiring a minimum of personnel time during the actual runs, because of practical limitations of time and personnel. The first method given serious consideration was that of actually counting the suspended particles with a photoelectric "counter." This method had proven useful in the study of inanimate aerosols, and our initial work with this method gave encouraging results. However, further investigations showed that our early results were in error, and that the instruments being employed were not actually "counting" the air-borne bacteria directly but responding to the background noises. Further study of the optical properties of air-borne droplets of water (which the bacteria most nearly resemble in physical properties) revealed the fact that such particles absorb more light than they scatter, and since the operation of the "counter" is based on light scattering, it was concluded that additional work with this instrument would not be justified.

The next method of direct counting involved the use of the extended-area thermal precipitator developed earlier in this work. Unfortunately, the small size of the bacterial particle after precipitation on a glass surface made it virtually impossible to perform routine counts in this manner. This method and other related methods (glass settling slides and inpactor slides) were discarded only after numerous attempts were made to stain the particles distinctively and to swell them for more easy identification.

Several other methods were investigated, such as the direct counting of wet-cell preparations or building-up the aerosol size prior to examination in a slit microscope, but none of them proved to be satisfactory for the

routine estimation of the total numbers of particles in the chamber. The failure of these attempts has emphasized the value of the taking of simultaneous aerial and settling samples (based on viability) during dynamic runs as a method of characterizing the physical properties of the airborne bacterial particles.

E. Studies on the Aerial Concentration in the Chamber Inlet and Outlet

Intermittently during the dynamic-chamber runs samples were taken from the inlet and outlet of the chamber as well as from the chamber itself. These were taken during the earlier studies when the chamber was being checked for uniformity of distribution of the bacterial aerosol, and only dynamic runs were made. After collecting these data, it was found that comparing the inlet concentration to the chamber concentration and the inlet concentration to the outlet concentration gave reproducible ratios as long as the runs were made at the same relative humidity, but not when the runs were made at another humidity. Our final studies on temperature and humidity have resulted in information which seems to offer adequate explanation of this situation. In Table XVI ratios of aerial concentration of bacterial particles, as described above, are listed for several humidities at 76° F. Assuming that these ratios are the result of the unfavorable action of a particular humidity causing the numbers of viable bacteria in the air to decrease, the proper k value for that humidity was estimated by interpolation from the temperature-humidity studies in section III. A (kt) value was calculated for each of the values listed in Table XVI by substitution in the equation

$$kt = \log n_o - \log n.$$

In each case the value for n_o was that for the inlet concentration.

Employing the proper k for the particular relative humidity, values for t_c (the apparent time lapse between the inlet and the chamber) and t_x (the apparent time lapse between the inlet and the outlet) were obtained. The resultant figures, expressed as time in minutes, are shown in Table XVII.

Although there is very poor agreement between the values enumerated in the Table XVII the conclusion seems warranted that the differences in numbers occuring between the inlet, chamber and outlet are primarily due to the continuing effect of the death rate typical of the particular humidity under study. Further, the values shown for the average apparent time lapse between the inlet, chamber and out are consistent with the known characteristics of chamber operation. The chamber is operated dynamically at approximately one-chamber volume per minute, and it would therefore be expected that the apparent time lapse between the inlet and the outlet would be identical with the time required for equilibrium, which in this case is approximately 4.5 minutes.

It is therefore concluded that the observed differences in numbers of air-borne bacteria between samples taken at the inlet, chamber and outlet are primarily caused by the same unfavorable effect of humidity that has been demonstrated in static runs,and not caused by any special or different effect related to chamber operation. In essence, the above information shows that it is possible to account for virtually all the bacteria which enter the chamber.

F. Cultural Methods

The cultural methods described in section I have been selected because they meet certain needs of this type of study. Thus, the stock culture for use in producing the bacterial aerosol is made in 0.3 per cent

beef-extract broth because this is a common cultural medium, and because excellent growth can be obtained even though the concentration of solids is low. Other media might be more suitable but it was not felt that the primary emphasis of this study was the selection of optimum media, but rather the selection of suitable and useful media. The same argument applies to the use of gelatin and agar plates and the impinger fluid. However, the temperatures at which the test organism, S. marcescens was grown were determined to be the optimum for the purposes at hand. Thus, a 40-hour-old culture incubated at 30° C yielded the most consistent results of any culture age or incubation temperature studied. These conditions, once established, have been standardized and constitute a fixed routine.

The poured-agar plates and agar plates from settling samples are incubated at 37° C because lower temperatures of incubation produced too many surface-spreading colonies, thereby making counting difficult. A temperature lower than 37° C is required for the production of the typical red pigment in the colonies (20° C optimum). Pigmentation progresses rapidly as soon as the plates are removed from the 37° C incubator, and within a few hours all colonies show definite coloration.

V. SIGNIFICANT ACCOMPLISHMENTS

Two significant accomplishments have resulted from the work carried out during the period covered by this report. The first and most important has been the completion of studies leading to the development of a reproducible technique for the evaluation of aerial bactericides. The methods selected for this technique have proved to be reproducible under a variety of conditions over a period of approximately a year. It is believed the technique developed will be acceptable to most workers, and if applied in those centers interested in aerobacteriology, it will be a material aid in the production of consistent and reproducible results.

The second significant accomplishment resulting from the work reported herein has been the application of the technique developed for the evaluation of aerial bactericides to a study of the effect of temperature and humidity on the survival of single-bacterium particles of air-borne S. marcescens. The results of this study have yielded more detailed and specific information as to the effect of temperature and humidity on an air-borne organism than previously reported; in general, these results have been consistent with those obtained by other workers.

VI. PLANS FOR NEXT YEAR

A request for further funds is planned, to enable the collection of sufficient data to fulfill the original objectives of the projected work. These objectives include a study of the effects of small amounts of a wide range of chemicals in the air on various bacteria, a correlation of aerial bactericidal effects with molecular properties of the bactericide and a development of a scale of bactericidal effectiveness for chemical vapors. Having completed the selection and proving of methods for this study, and having investigated the effect of temperature and humidity on air-borne S. marcescens, work is now under way on the evaluation of candidate aerial bactericides. It is hoped that these objectives can be attained within a year at the rate of effort proposed.

VII. APPENDIX

TABLE I

AVERAGES* OF SIMULTANEOUS AIR SAMPLES

Ten Samples**		Three Samples**	
Run No.	Average and S.D.	Run No.	Average and S.D.
47-A	450 ± 38	428-P1	400 ± 45
47-P	420 ± 33	428-P2	300 ± 18
48-A	52 ± 9	432-A1	174 ± 17
416-A	42 ± 7	423-A2	126 ± 8
48-P1	33 ± 6	428-A2	74 ± 7
48-P2	26 ± 5	51-P1	38 ± 3

*The values given are the averages of actual plate counts.

**Samples taken for a period of 35 minutes.

TABLE II

AVERAGES OF SETTLING PLATES

Time in Minutes	Run No. 429-P1 Average and S.D. for Four Plates	Run No. 51-P12 Average and S.D. for Four Plates
0-5	193 ± 19	266 ± 60
10-15	185 ± 25	312 ± 70
20-25	202 ± 45	244 ± 25
30-35*	208 ± 33	330 ± 70
Ave. 0-35	197 ± 16	288 ± 26
50-55	202 ± 25	305 ± 65
60-65	200 ± 30	252 ± 23
70-75	223 ± 34	314 ± 39
80-85	195 ± 19	295 ± 65
Ave. 50-85	205 ± 25	291 ± 39

*Atomizer refilled at this time.

TABLE III

RESULTS OBTAINED FROM AVERAGE DATA FOR RUNS AT TEMPERATURES OF 60°-70° F

Condition of Run			Culture	Number of	Dynamic Runs					Static Runs	
R.H.	D.B.*	D.P.**	Count	Replicates	Conc/L	Settling	Rate of Fall	Apparent Diameter	k_f	k_t	Net k Due to Death
(%)	(°F)	(°F)	(10^7)			(min/1,000 cm^2)	(cm/min)	(μ)			
20	66	25	58	3	2,600	3,120	1.20	2.58	0.00430	0.0173	0.0130
23	66	27	66	2	2,700	3,260	1.21	2.58	0.00432	0.0261	0.0218
35	66	38	55	3	1,600	2,100	1.31	2.70	0.00467	0.0306	0.0259
45	65	44	67	1	1,800	2,900	1.62	3.00	0.00580	0.0227	0.0169
48	64	44	53	3	1,800	2,700	1.50	2.87	0.00535	0.0207	0.0154
50	64	45	53	3	1,500	2,300	1.53	2.91	0.00545	0.0189	0.0135
50	67	48	69	3	1,200	2,200	1.83	3.17	0.00655	0.0252	0.0187
60	65	50	57	3	1,600	3,400	2.13	3.43	0.00761	0.0341	0.0265
74	68	59	63	2	2,300	4,700	2.04	3.33	0.00730	0.0311	0.0238
75	68	60	51	1	2,100	4,300	2.05	3.34	0.00730	0.0190	0.0117
80	70	64	49	3	2,900	5,600	1.93	3.26	0.00690	0.0102	0.0033
82	66	60	75	1	3,270	7,900	2.42	3.72	0.0085	0.0133	0.0048
92	70	68	70	3	3,100	6,100	1.97	3.30	0.00704	0.0131	0.0060

*Dry bulb.

**Dew point.

TABLE IV

RESULTS OBTAINED FROM AVERAGE
DATA FOR RUNS AT 80° F

Condition of Run			Culture	Number of	Dynamic Runs					Static Runs	
R.H.	D.B.*	D.P.**	Count	Replicates	Conc/L	Settling	Rate of Fall	Apparent Diameter	k_f	k_t	Net k Due to Death
(%)	(°F)	(°F)	(10^7)			(min/1,000 cm^2)	(cm/min)	(μ)			
15	80	27	45	2	1,280	1,950	1.51	2.9	0.0054	0.0134	0.008
23	80	39	53	4	2,340	3,700	1.58	3.0	0.0057	0.0448	0.0391
28	80	43	--	2	1,460	2,400	1.64	3.0	0.0059	0.0452	0.0393
30	80	45	70	6	1,050	3,600	3.42	4.3	0.0122	0.0485	0.0363
32	80	47	56	3	327	682	2.09	3.4	0.0075	0.0605	0.0530
34	80	48	62	3	660	1,180	1.80	3.1	0.0064	0.0580	0.0516
34	80	48	65	3	1,180	5,260	4.45	4.9	0.0159	0.0618	0.0459
37	80	51	54	3	870	3,400	3.98	4.6	0.0138	0.0950	0.0812
38	80	52	55	2	660	2,500	3.78	4.6	0.0135	0.1095	0.0960
45	80	57	65	3	865	3,000	3.48	4.4	0.0124	0.164	0.152
50	80	60	60	6	1,275	2,165	1.70	3.1	0.0061	0.20	0.19
60	80	65	69	3	2,230	3,640	1.63	3.0	0.0058	0.138	0.132
70	80	70	59	3	1,250	2,175	1.74	3.1	0.0062	0.154	0.148
90	80	77	55	3	1,060	3,500	3.30	4.3	0.0118	0.1160	0.1042
95	82	80	50	6	740	2,400	3.24	4.2	0.0116	0.052	0.0404

*Dry bulb
**Dew point

TABLE V

RESULTS OBTAINED FROM AVERAGE
DATA FOR RUNS AT 90° F

Condition of Run			Culture	Number of	Dynamic Runs					Static Runs	
R.H.	D.B.*	D.P.**	Count	Replicates	Conc/L	Settling	Rate of Fall	Apparent Diameter	k_f	k_t	Net k Due to Death
(%)	(°F)	(°F)	(10^7)			(min/1,000 cm^2)	(cm/min)	(μ)			
12	90	31	54	3	780	725	0.94	2.37	0.00332	0.0455	0.042
23	92	48	62	3	175	277	1.58	2.98	0.00565	$\geq$0.5	0.5
24	92	50	60	3	205	400	1.95	3.28	0.00695	$\geq$0.5	0.5
50	91	70	65	3	472	510	1.08	2.45	0.00385	$\geq$0.5	0.5
75	91	82	54	3	430	955	2.22	3.50	0.00795	$\geq$0.5	0.5
95	92	90	60	3	100	500	5.00	5.20	0.0175	0.106	0.089

*Dry bulb

**Dew point

TABLE VI

EFFECT OF HUMIDITY ON THE NUMBER OF VIABLE AIR-BORNE
S. MARCESCENS IN THE CHAMBER AT 60°-70° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	Average Number per Liter**
20	66	25	3	4,550
23	66	27	3	4,150
35	66	38	3	2,970
45	65	42	1	2,600
48	64	44	3	3,400
50	64	45	3	2,770
50	67	48	3	2,500
60	65	50	3	2,870
74	68	59	3	3,760
75	68	60	1	4,050
80	70	64	3	5,980
82	66	60	3	4,380
92	70	68	3	4,420

*Dynamic runs.

**In terms of 100 x 10^7/ml culture

TABLE VII

EFFECT OF HUMIDITY ON THE NUMBER OF VIABLE AIR-BORNE S. MARCESCENS IN THE CHAMBER AT 80° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	Average Number per Liter**
15	80	27	2	2,850
23	80	39	4	4,400
30	80	45	6	1,500
32	80	47	3	580
34	80	48	3	1,070
34	80	48	3	1,820
37	80	51	3	1,600
38	80	52	2	1,200
45	80	57	3	1,330
50	80	60	6	2,120
60	80	65	3	3,200
70	80	70	3	2,210
90	80	77	3	1,930
95	82	80	6	1,480

*Dynamic runs

**In terms of 100 x 10^7 ml culture

TABLE VIII

EFFECT OF HUMIDITY ON THE NUMBER OF VIABLE AIR-BORNE *S. MARCESCENS* IN THE CHAMBER AT 90° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	Average Number per Liter**
12	90	31	3	1,450
23	92	48	3	280
24	92	50	3	340
50	91	70	3	730
75	91	82	3	800

*Dynamic runs

**In terms of 100 x 10^7/ml culture

TABLE IX

EFFECT OF HUMIDITY ON THE NUMBER OF AIR-BORNE
S. MARCESCENS SETTLING IN THE CHAMBER AT 60°-70° F

Condition of Run*			Number of Replicates	Number Settling per Minute per 1,000 cm^{2}**
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)		
20	66	25	3	5,350
23	66	27	3	4,950
35	66	38	3	3,820
45	65	44	1	4,320
48	64	44	3	5,100
50	64	45	3	4,350
50	67	48	3	3,200
60	65	50	3	5,950
74	68	59	2	7,450
75	68	60	1	8,400
80	70	64	3	11,400
82	66	60	1	10,500
92	70	68	3	8,700

*Dynamic runs

**In terms of 100 x 10^{7}/ml culture

TABLE X

EFFECT OF HUMIDITY ON THE NUMBER OF AIR-BORNE S. MARCESCENS SETTLING IN THE CHAMBER AT 80° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	Number Settling per Minute per 100 cm^2**
15	80	27	2	4,230
23	80	39	4	7,000
30	80	45	6	5,150
32	80	47	3	1,220
34	80	48	3	1,900
34	80	48	3	8,100
37	80	51	3	6,300
38	80	52	2	4,550
45	80	57	3	4,600
50	80	60	6	3,620
60	80	65	3	5,250
70	80	70	3	3,700
90	80	77	3	6,350
95	82	80	6	4,800

*Dynamic runs

**In terms of 100 x 10^7/ml culture

TABLE XI

EFFECT OF HUMIDITY ON THE NUMBER OF AIR-BORNE
S. MARCESCENS SETTLING PER MINUTE IN THE CHAMBER AT 80° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	Number Settling per Minute per 1,000 cm^2**
12	90	31	3	1,340
23	92	48	3	435
24	92	50	3	670
50	91	70	3	785
75	91	82	3	1,770

*Dynamic runs.

**In terms of 100 x 10^7/ml culture

TABLE XII

EFFECT OF HUMIDITY ON THE TIME REQUIRED FOR DEATH OF 90 PER CENT OF AIR-BORNE S. MARCESCENS AT 60°-70° F

Condition of Run*				
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)	Number of Replicates	t_{90} (minutes)
20	66	25	3	77
23	66	27	3	46
35	66	38	3	39
45	65	44	1	59
48	64	44	3	65
50	64	45	3	74
50	67	48	3	54
60	65	50	3	38
74	68	59	2	42
75	68	60	1	86
80	70	64	3	304
82	66	60	1	208
92	70	68	3	166

*Static runs

TABLE XIII

EFFECT OF HUMIDITY ON THE TIME REQUIRED FOR DEATH OF 90 PER CENT OF AIR-BORNE S. MARCESCENS AT 80° F

Condition of Run*			Number of Replicates	t_{90}
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)		(minutes)
15	80	27	2	125
23	80	39	4	26
28	80	43	2	26
30	80	45	6	28
32	80	47	3	19
34	80	48	3	19
34	80	48	3	22
37	80	51	3	12
38	80	52	2	10
45	80	57	3	7
50	80	60	6	5
60	80	65	3	7
70	80	70	3	7
90	80	77	3	10
95	82	80	6	25

*Static runs

TABLE XIV

EFFECT OF HUMIDITY ON THE TIME REQUIRED FOR DEATH OF 90 PER CENT OF AIR-BORNE S. MARCESCENS AT 90° F

Condition of Run*			Number of Replicates	t_{90}
Relative Humidity (%)	Dry Bulb (°F)	Dew Point (°F)		(minutes)
12	90	31	3	25
23	92	48	3	2
24	92	50	3	2
50	91	70	3	2
75	91	82	3	2
95	92	90	3	9

*Static runs

TABLE XV

REPRESENTATIVE DATA COMPARING COUNTS FROM GELATIN PLATES WITH THOSE FROM AGAR PLATES

Count on Gelatin Plate (30 min. exposure) (number/minute)	Count on Agar Plate (1 min. exposure) (number/minute)	Agar Plate Count Corrected for Overlapping*	Agar/Gelatin x 100
33	28	28	85
46	36	38	82
62	65	67	108
75	64	66	88
148	131	149	101
153	110	120	79
164	141	161	98
227	191	226	99
304	254	306	101
341	250	302	89
		Average	93

*Mack, C., "The Effect of Overlapping in Bacterial Counts of Incubated Colonies," Biometrika 40, 220-2 (1953).

TABLE XVI

RATIO OF THE NUMBER OF AIR-BORNE BACTERIA AT INLET, CHAMBER AND OUTLET AT 76° F

Relative Humidity (%)	Ratio of Inlet/Chamber	Ratio of Inlet/Outlet
45	0.45	0.28
70	0.68	0.53
74	0.68	0.62
78	0.78	0.67
85	0.89	0.57

TABLE XVII

APPARENT TIME LAPSES BETWEEN INLET, CHAMBER AND OUTLET AT 76° F

Relative Humidity (%)		Time Lapses Between Inlet and Chamber (minutes)	Time Lapses Between Inlet and Outlet (minutes)
45		2.23	4.45
70		2.45	4.95
74		1.76	3.67
78		2.63	5.50
85		3.06	6.07
	Average	2.43	4.92

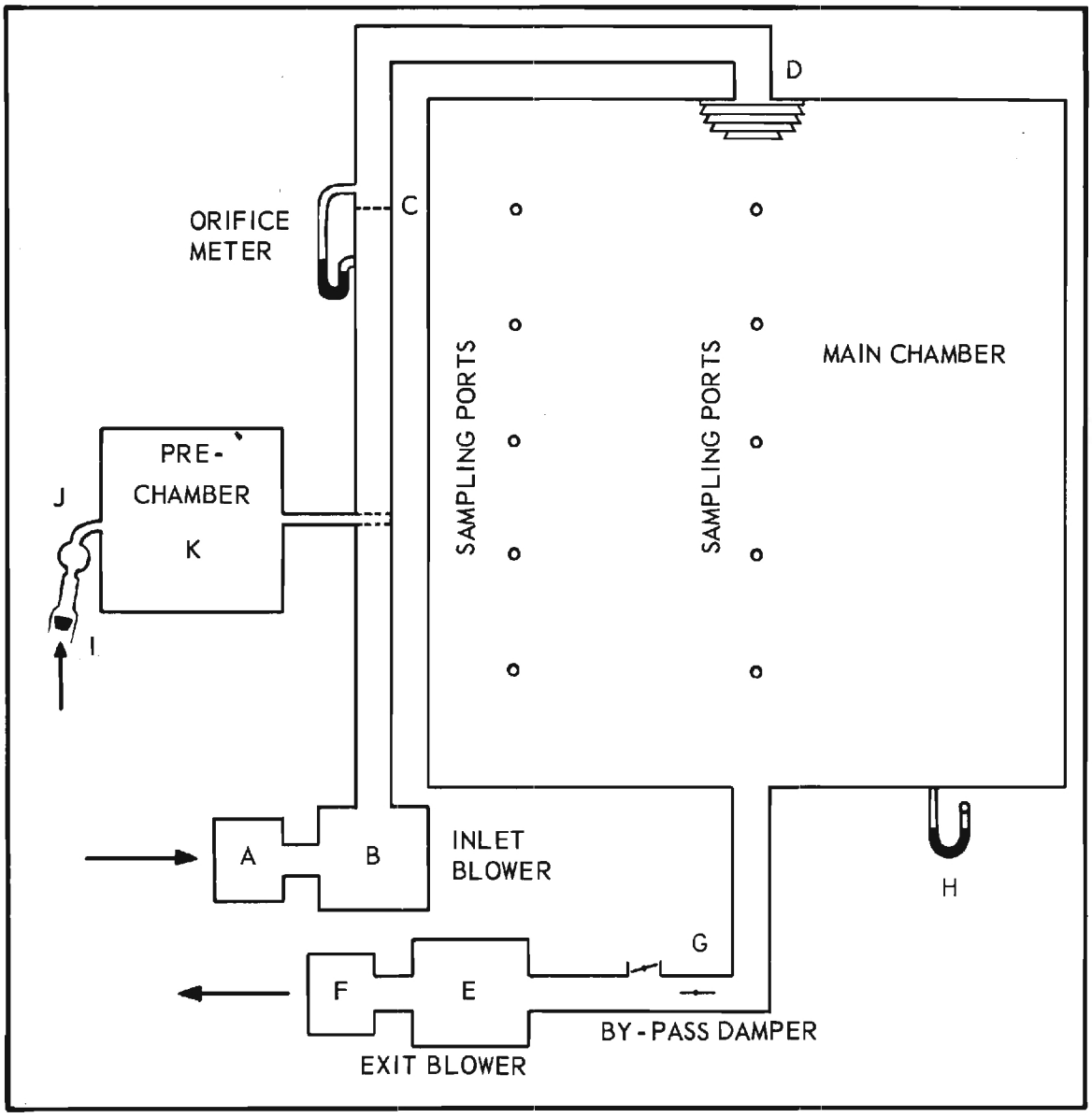

Figure I. Schematic Drawing of Chamber and Atomizing Equipment.

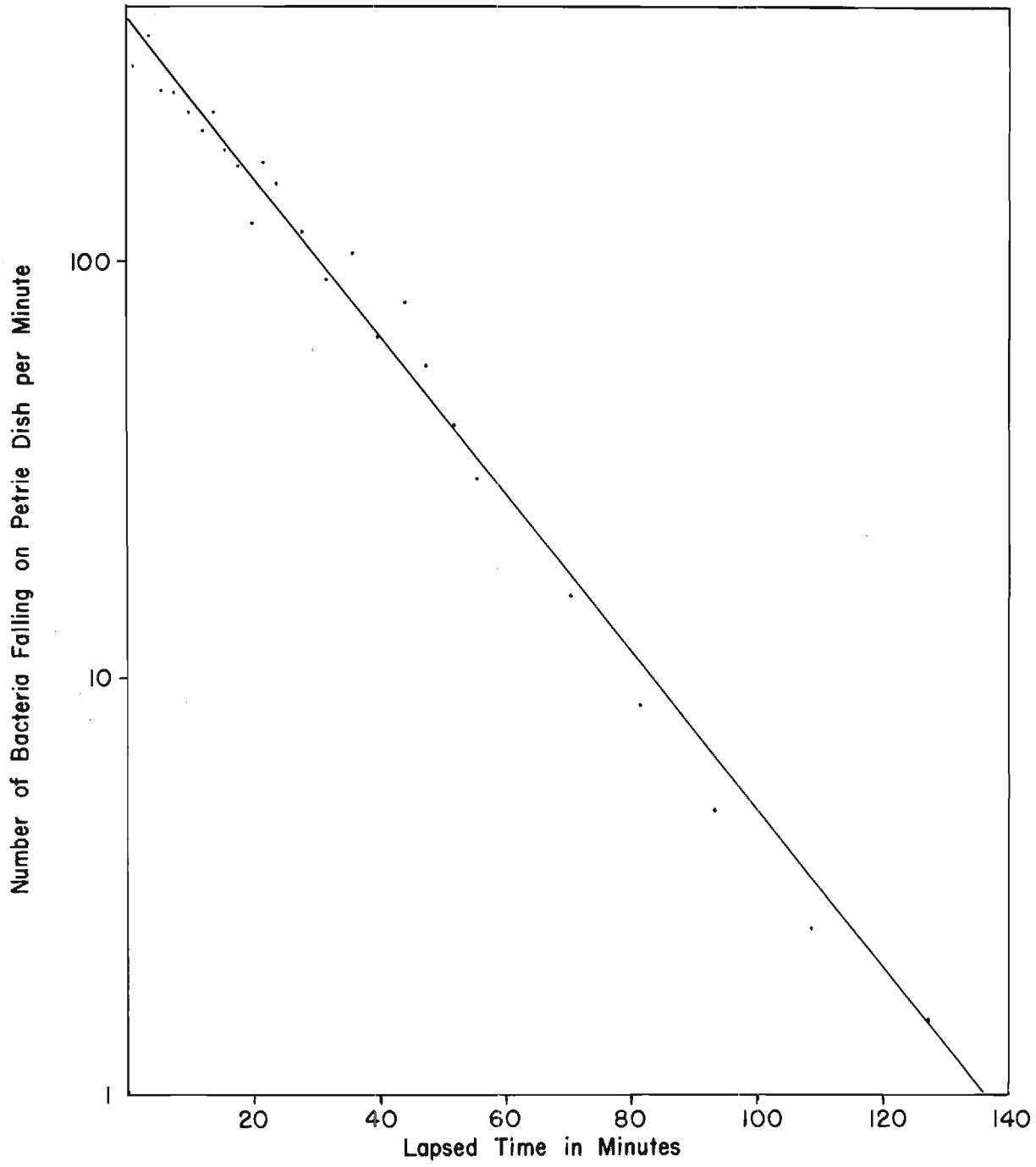

Figure 2. Results of Static Run at 75 Per Cent Relative Humidity and 68°F.

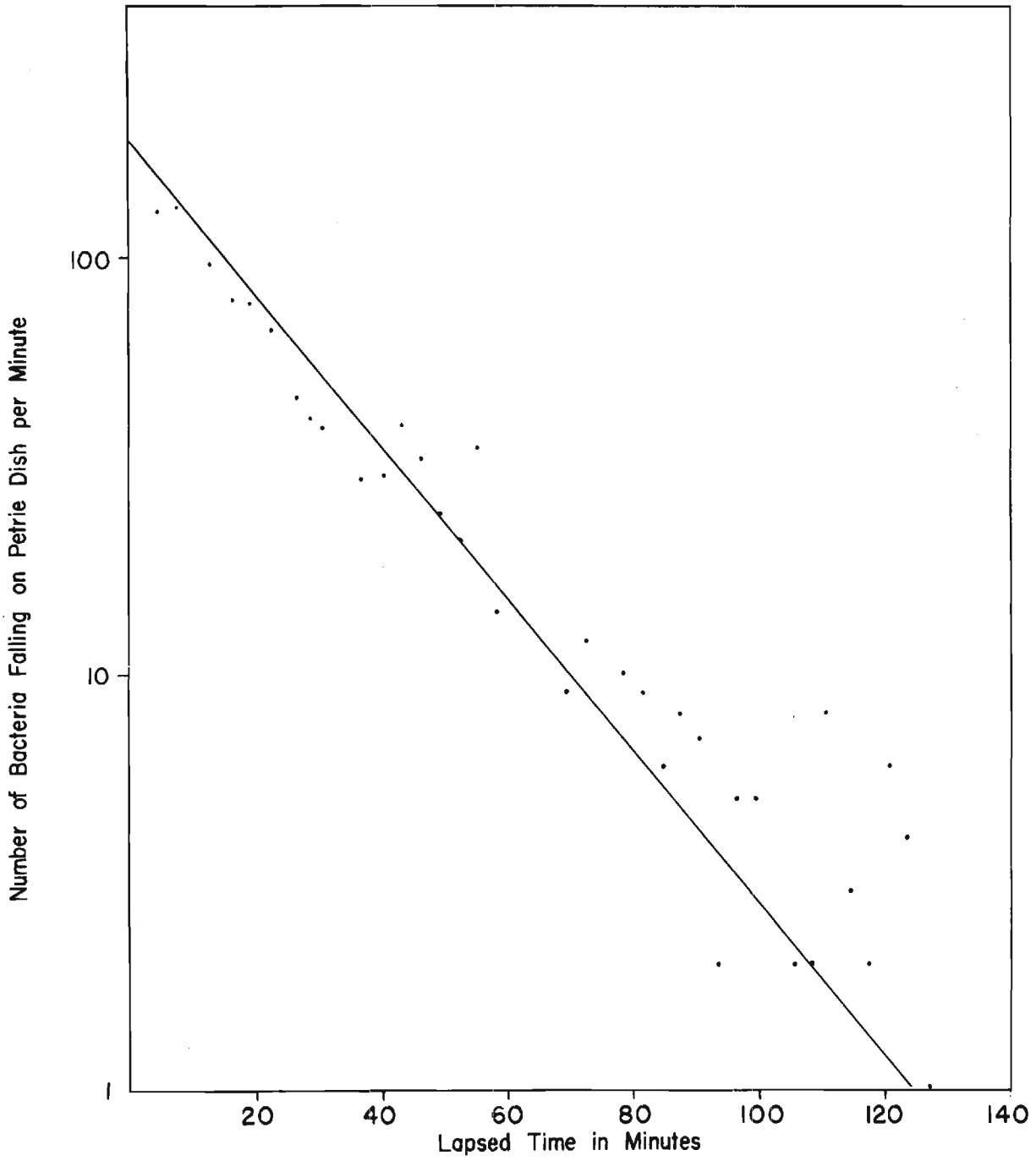

Figure 3. Results of Static Run at 45 Per Cent Relative Humidity and 68°F.

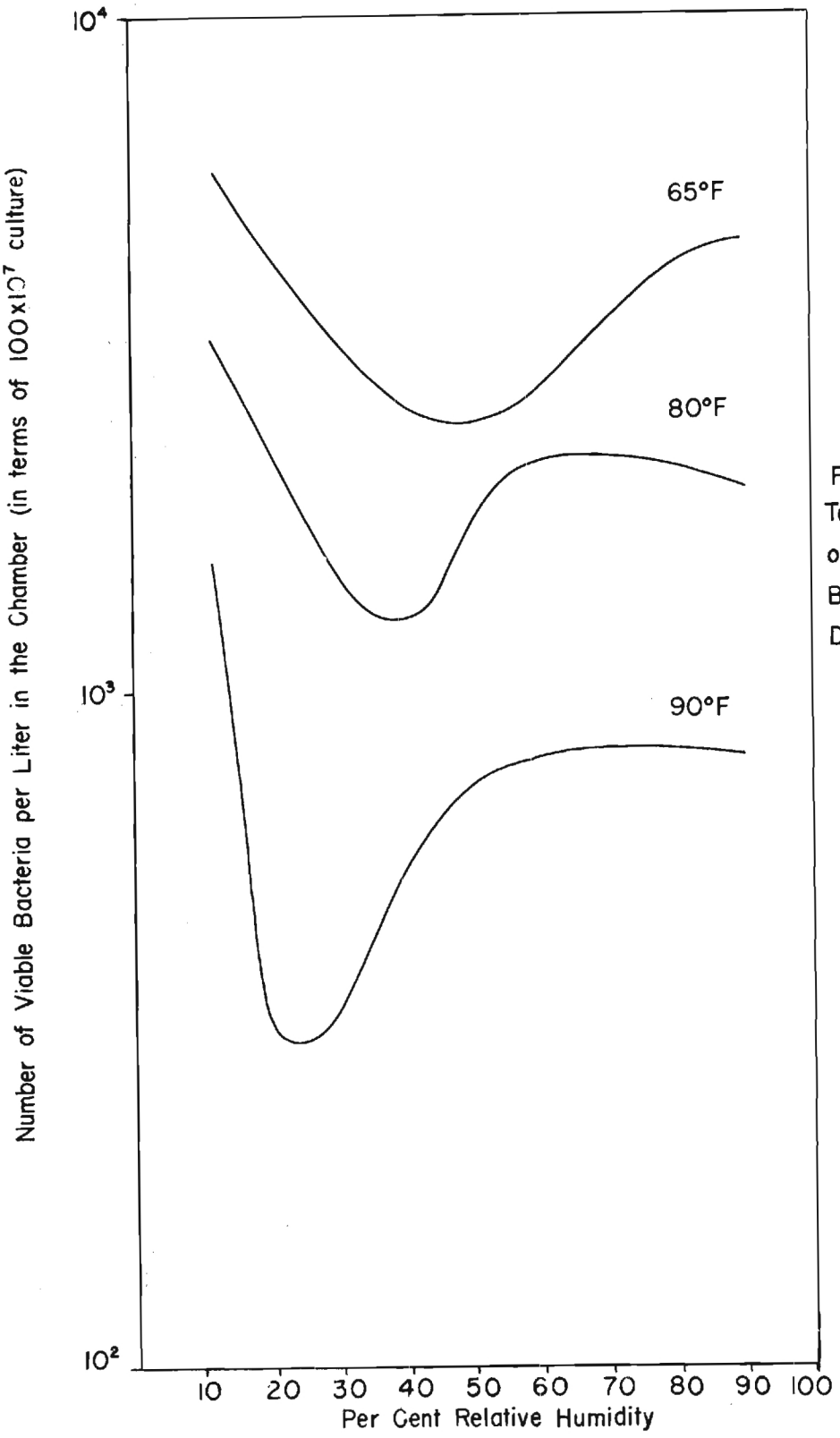

Figure 4. Effect of Temperature and Humidity on the number of Bacteria in the Chamber During Dynamic Runs.

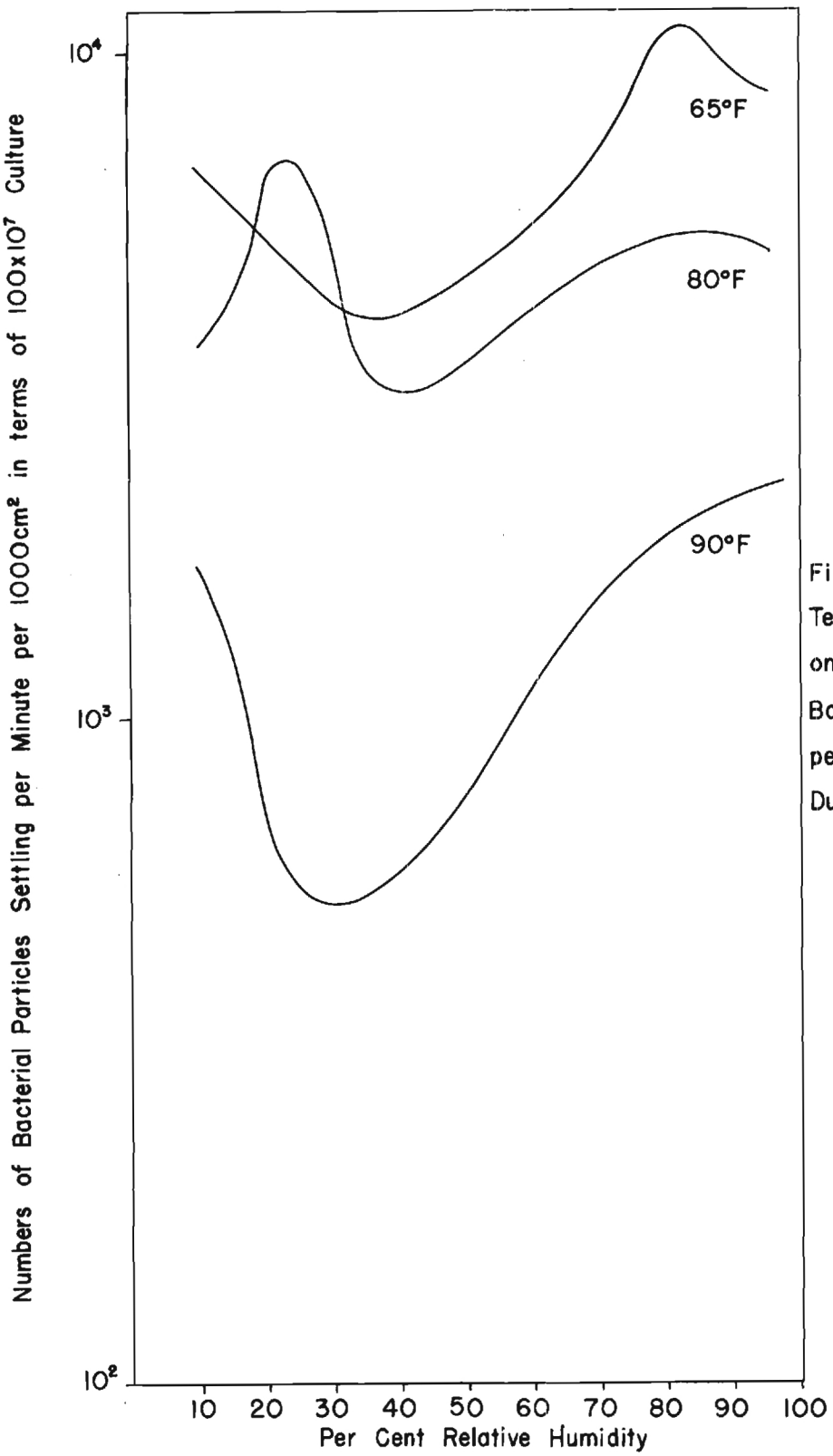

Figure 5. Effect of Temperature and Humidity on the Number of Bacterial Particles Settling per Minute per 1000cm² During Dynamic Runs.

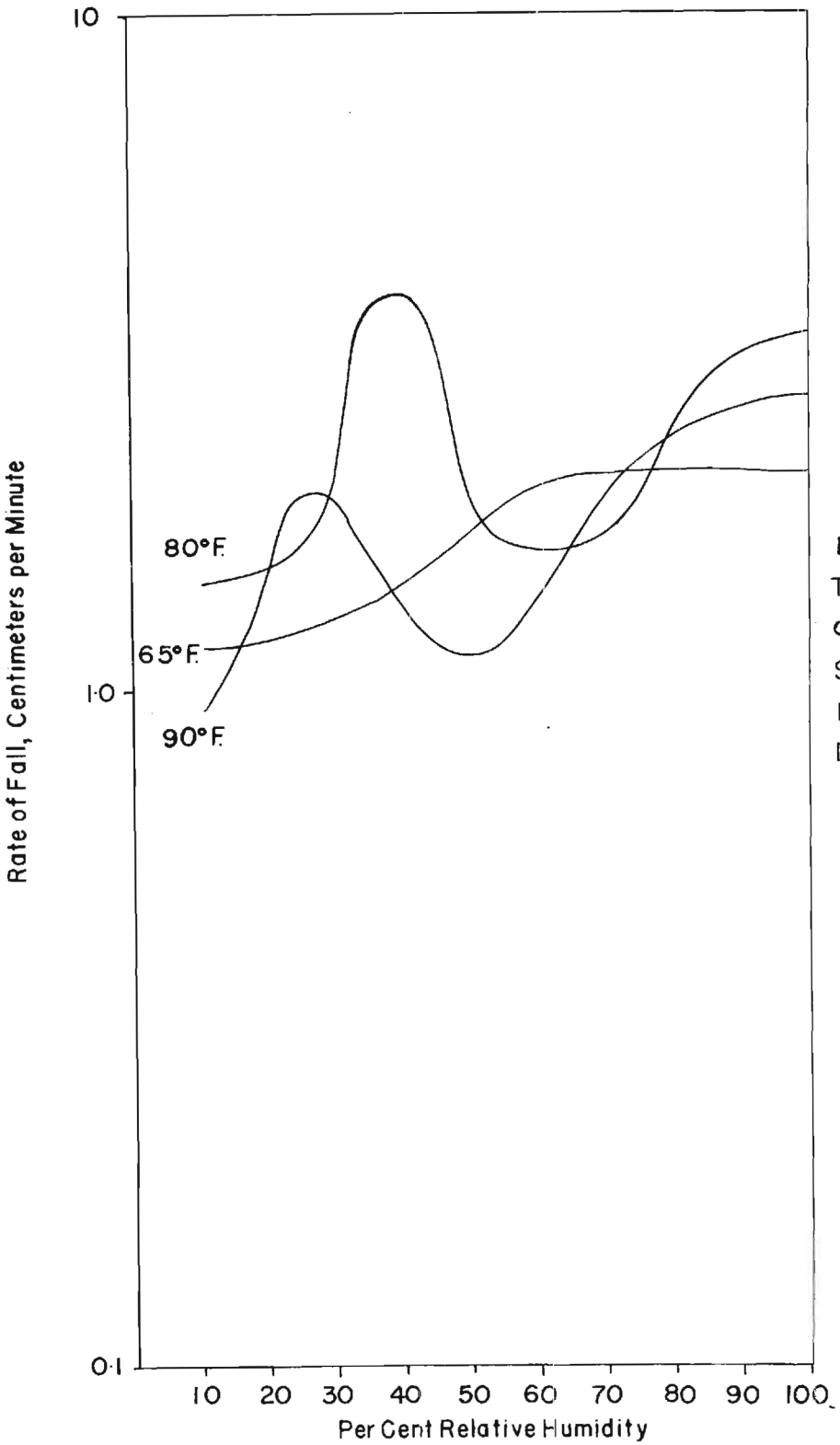

Figure 6. Effect of Temperature and Humidity on the Apparent Rate of Settling of Bacterial Particles During Dynamic Runs.

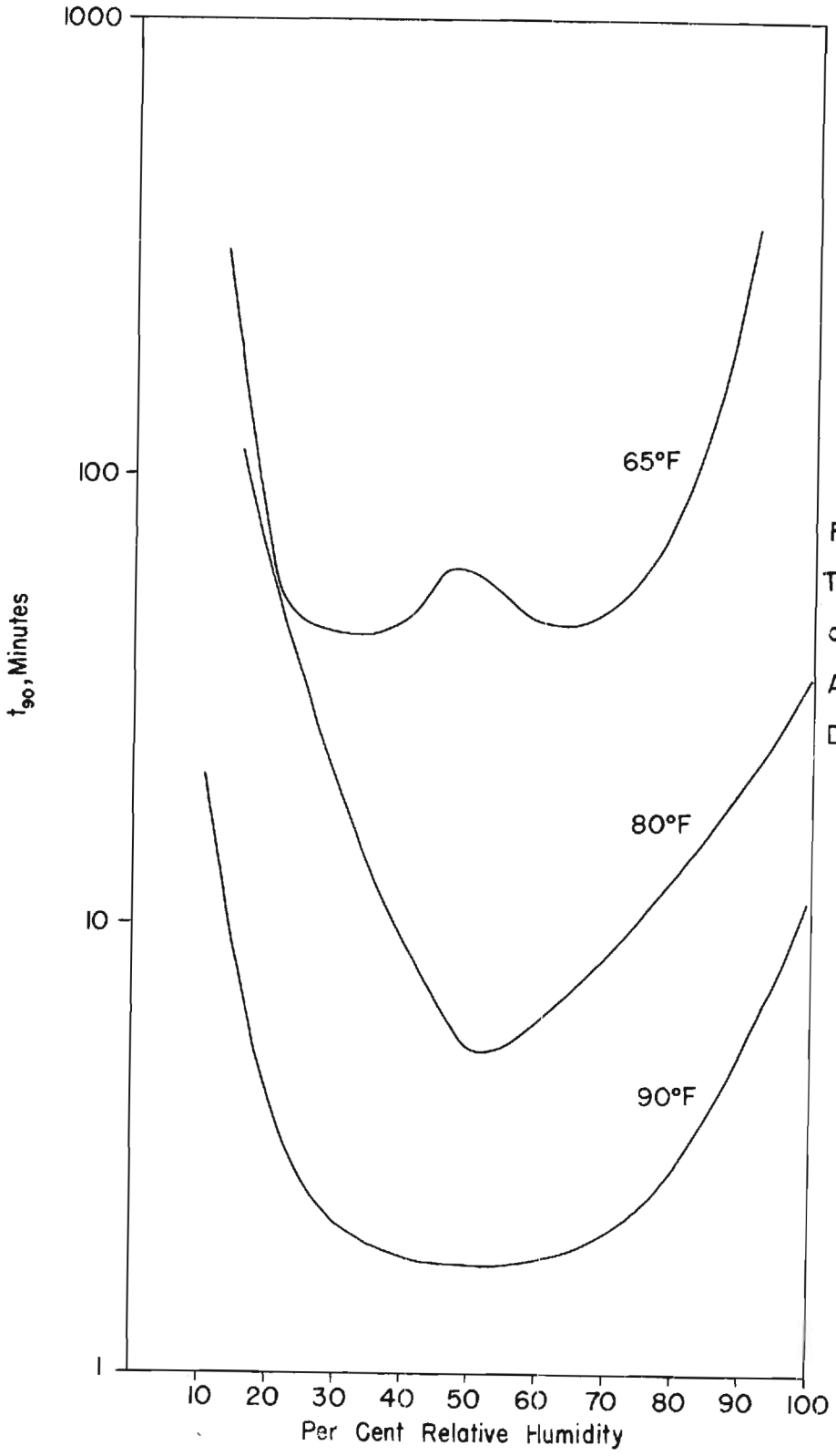

Figure 7. Effect of Temperature and Humidity on the Viability of Airborne S. marcescens During Static Runs.