SUMMARY

A key based on paper chromatography and bioautography is proposed for differentiating most of the gram positive inhibiting antibiotics produced by *Bacillus*, as well as polypeptidic antibiotics produced by other microorganisms.

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Studies of Aerosols with a Simple Cloud-Chamber Technic[1]

I. The Evaluation of a Technic for the Rapid and Convenient Determination of the Survival of Air-Borne Microorganisms

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Since the realization that the atmosphere could serve as a vector for the transmission of disease-producing agents, many investigations have been performed to determine the microbial flora of air as well as the environmental factors which affect survival. Bacteriological evaluation of the latter presents a special problem in instrumentation and technic.

The purpose of this investigation was to develop a simple, small-scale, cloud chamber technic for the study of bacterial aerosols as influenced by physical environmental conditions such as relative humidity, temperature, and solar radiation. To accomplish this, a small portable chamber was desired, one that was simple in design, convenient for adjustment of or exposure to variations in physical conditions and capable of providing reproducible results. The practicability of such equipment was suggested by the work of Van den Ende *et al.* (1948), who used small, round-bottom, quartz flasks to study the bactericidal effect of ultraviolet light on bacterial aerosols.

For the analysis of the concentrations of air-borne bacteria, a number of air-sampling technics (based on two methods of collection) have generally been employed by various investigators. The first includes those devices which collect organisms in a liquid and break up any clumps of bacterial cells, such as the bubbler-pump method of Wheeler *et al.* (1941) and the atomizer-bubbler apparatus of Moulton *et al.* (1943). The second type of sampler impinges the bacteria directly onto a solid medium without attempting to break up clumps and is represented by the Wells' air centrifuge (1933), the funnel device of Hollaender and DallaValle (1939), the slit sampler developed by Bourdillon *et al.* (1941), the sieve device of DuBuy and Crisp (1944) and, more recently, the application of the molecular filter membrane by Goetz (1953). Another recent technic used for analyzing aerosols is the electronic counter developed by Gucker and O'Konski (1949).

In the present investigation, a syringe-dilution method (a liquid-collection technic) and the slit-sampler method (a solid-impingement technic) were evaluated for their suitability in the analysis of static bacterial aerosols produced in the small cloud chamber to be described. These technics have been adapted from those investigated and recommended by personnel of the Chemical Corps, Camp Detrick.

EXPERIMENTAL METHODS

The cloud-chamber technic employed in our studies consisted essentially of the production of a bacterial aerosol in an inverted, one-liter, round-bottom flask (aerosol chamber) and the transfer of a portion of this aerosol to another inverted one-liter flask (transfer chamber) from which analyses of bacterial survival were made. This type of chamber was selected because

[1] This investigation was performed under contract for the Chemical Corps, Camp Detrick, Frederick, Maryland.

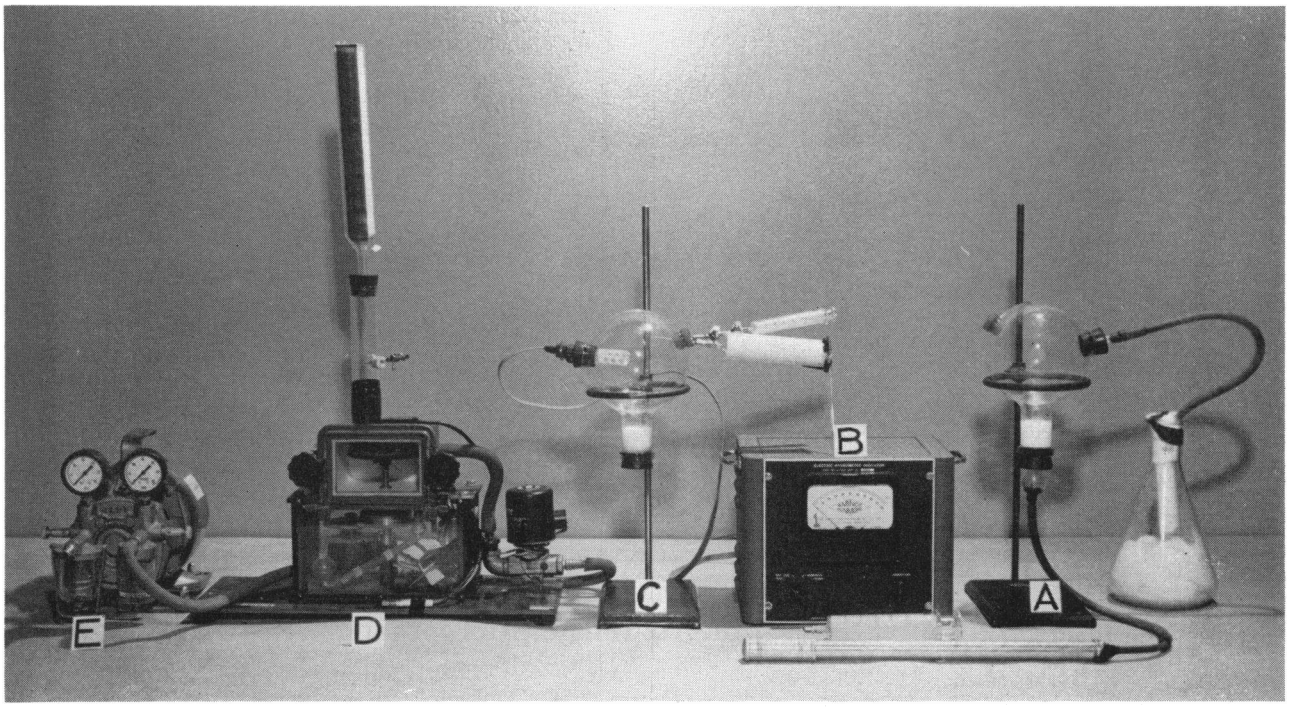

FIG. 1. Equipment employed in the small cloud-chamber technic: (A) Aerosol flask in which original cloud is produced. (B) Aminco Electric Hygrometer by which temperature and humidity measurements are made. (C) Transfer flask from which aerosol samples are withdrawn for analysis. (D) Slit sampler, Casella and Co., London. (E) Vacuum pump.

of the adaptability offered for studying the effect of such physical environmental conditions as relative humidity, temperature, and solar radiation. The equipment employed in the simple cloud-chamber technic is shown in figure 1, together with the slit sampler, the impingement device used in one of the sampling methods studied.

The test bacterium employed in all studies was the Webb strain of *Serratia marcescens*. Stock cultures were carried on slants of the synthetic medium developed by Bunting (1940) which allows for better maintenance of pigmentation. Cultures were transferred weekly, incubated at 27 C for 24 hours, and then stored at 4 to 6 C for the remainder of the week. In preparing cell crops for aerosolization, 25 ml of trypticase-soy broth contained in a 250-ml Erlenmeyer flask were inoculated from the stock slant and incubated on a rotary-type shaker at 27 C for 24 hours. Following this incubation period, one ml from this culture was transferred to 100 ml of trypticase-soy broth in a one-liter Erlenmeyer flask which was also incubated on a shaker at 27 C for 24 hours. The resulting broth culture was centrifuged and reconstituted to one-tenth its original volume with a cell-suspending medium (0.85 per cent saline unless otherwise indicated). This "cell concentrate" (10^{11} to 10^{12} cells per ml) was employed as the standard cell suspension throughout these experiments.

The bacterial cloud was produced in the aerosol flask by 15 strokes of a hand-operated bicycle pump connected with a rubber hose to a DeVilbiss No. 44 nebulizer containing 3 ml of the "cell concentrate." This procedure was found to give reproducible concentrations of cells in the aerosols. No attempt was made to control the humidity in the aerosol flask (figure 1-A) because of the rapid rise in humidity as a direct result of aerosol production. Instead, a portion of the aerosol (50 or 100 ml) was transferred by means of a syringe to the transfer flask (figure 1-C), where the humidity could be more effectively controlled. Approximately 15 seconds elapsed during the transfer. Immediately after this operation, the first sample for bacterial analysis (zero time) was withdrawn from the transfer flask.

A wide range of relative humidities has been obtained by the use of several humidity control solutions placed in the necks of the inverted transfer flasks. Before beginning the experiments, the flasks were equilibrated until the desired humidity was established. Temperature and humidity measurements inside the flasks were made by means of an Aminco Electric Hygrometer[2] sensing element, which was inserted into a port in the side of each flask.

Two technics, the slit-sampler and syringe-dilution, were studied for the analysis of cell concentrations in the transfer flasks. In the slit-sampler method (see figure 1 for equipment), samples were removed at designated time intervals with a greased syringe (the volume of sample and time of sampling being determined by the rapidity of decay at various humidities) and expelled slowly into the glass adaptor tube on the

[2] American Instrument Company, Silver Spring, Maryland.

slit sampler. The introduction of the sample was accomplished during 25 seconds of the 30-second plate rotation period. Bacteria in the aerosol samples were impinged upon the surface of trypticase-soy agar plates revolving on the turntable of the sampler. These plates were incubated at 37 C for 24 hours, following which colonies were counted and decay rate calculations made from the data.

The syringe-dilution method made use of the pieces of equipment labeled A, B, and C in figure 1. In this technic, 5-ml samples were withdrawn from the transfer flask at designated time intervals in 10-ml syringes containing 5 ml of sterile 0.85 per cent saline. Syringes were equipped with stopcocks to prevent loss of aerosol during the shaking process. The syringes were shaken for one minute and the contents expelled into sterile test tubes from which appropriate dilutions were made and plated in trypticase-soy agar. The inoculated plates were handled in the same manner described for the slit-sampler technic.

The rate of decay of the aerosols, expressed as per cent per minute, was determined by the method of least squares, utilizing the linear regression calculation. The slope of the regression of the log count on time was found by the formula:

$$b = \frac{\Sigma (\log X)t - \dfrac{\Sigma (\log X)\Sigma t}{N}}{\Sigma t^2 - \dfrac{(\Sigma t)^2}{N}}$$

A correction factor[3] was applied to the slope in order to compensate for the slight dilution effect which accompanied the withdrawal of samples from the transfer chamber. The decay rate, per cent per minute, was then determined by multiplying the corrected slope (b) by 230.26.

Results and Discussion

To evaluate the slit-sampler and syringe-dilution technics, experiments were performed at two relative humidities, 30 per cent and 70 per cent. Saturated calcium chloride solution was employed to maintain the lower humidity of 30 per cent, and 28 per cent sulfuric acid solution, the higher humidity of 70 per cent. In the syringe-dilution method, 5-ml samples were taken throughout, whereas in the slit-sampler method, 10-ml samples were taken at 30 per cent humidity and 2.5-ml samples at 70 per cent humidity.

Figure 2 shows a comparison of the results obtained with the two methods. Very good agreement between the two methods was obtained at 70 per cent humidity, where mean decay rates of 4.29 per cent per minute with the slit-sampler method and 4.28 per cent per minute with the syringe-dilution method were obtained. At the lower humidity of 30 per cent (more specifically, 30 to 32 per cent humidity), a mean decay rate of 69.04 per cent per minute was obtained using the slit sampler and 80.10 per cent per minute with the syringe-dilution technic. This difference in results is not considered significant because, as will be reported later, a critical humidity range from approximately 25 to 40 per cent humidity has been established. In this range, major changes in decay rate values occur with minor changes in humidity.

The above results were calculated from data obtained from the following number of individually performed experiments covering a period of several months:

At 70 per cent humidity:
30 experiments (slit-sampler)
29 experiments (syringe-dilution)
At 30 per cent humidity:
18 experiments (slit-sampler)
15 experiments (syringe-dilution)

The results obtained by both the slit sampler and the syringe-dilution technics were in close agreement.

From the standpoint of ease and speed of performance, the slit-sampler technic offers several advantages. In this technic, samples are inoculated directly onto plates without further dilution. In order to obtain countable plates, a number of preliminary adjustments must first be made in the initial concentration of cells in the transfer flask and the volume of sample to be taken. However, once these adjustments have been made, this information can be employed in all subsequent experiments of the same type and a large number of aerosols can be analyzed in a given time. Thus, with the slit-sampler technic a larger number of determinations may be made during one day's work, and any variations which may be present from day to day are minimized.

With the syringe-dilution technic, the only preliminary manipulation necessary is that of securing a sufficiently high initial concentration of cells in the

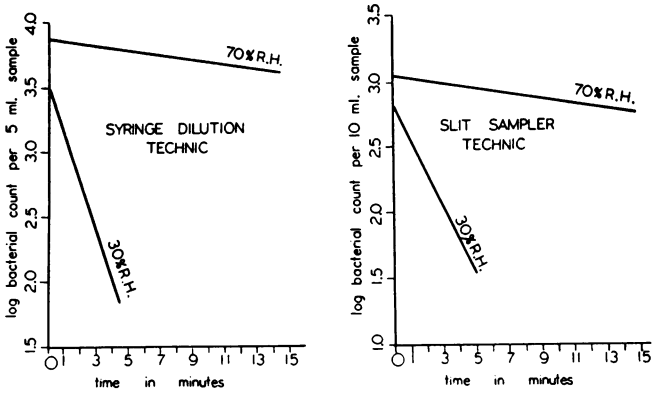

Fig. 2. Comparison of results of the two sampling technics, showing survival curves of aerosols of *Serratia marcescens* (Webb) at two relative humidities.

[3] We are indebted to Dr. G. S. S. Ludford of the Department of Mathematics and the Institute of Fluid Dynamics, University of Maryland, for the derivation of this correction factor.

transfer flask, since dilutions of the aerosol samples may be prepared following the sampling process. However, the preparation of dilutions and the subsequent plating operation consume much time and materials and greatly reduce the number of experiments which can be performed in a given time. On the other hand, specialized equipment is not required for the performance of the syringe-dilution technic.

The aspect of adaptability must also be considered in evaluating these two technics. It is desirable that the method be such as to be readily applicable to the study of the effect of such physical environmental conditions as relative humidity, temperature, and solar radiation. For instance, the mobility and compactness of the slit-sampler equipment and the continuity of this technic render this method more suitable for solar radiation studies where equipment must be transported and set up at a number of locations.

Thus, after comparing the two methods of aerosol analysis as to their relative accuracy and dependability, the ease and speed of performance and their adaptability to the study of a number of conditions, the slit-sampler technic was selected as the method of choice in preference to the syringe-dilution technic. It was felt that with the slit-sampler technic, results could be secured more quickly once preliminary adjustments were determined, and with the same degree of reproducibility.

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SUMMARY

A simple, small-scale, cloud-chamber technic has been evaluated for the study of bacterial aerosols under a variety of conditions. The slit-sampler and syringe-dilution technics, two methods of analyzing cell concentrations in these aerosols, have been studied and evaluated. Although comparable results were obtained by both technics, the slit-sampler method was found more convenient for the study of bacterial aerosol stability under the conditions employed in this investigation. This technic provides for rapid analysis of aerosol samples with the same degree of reproducibility as found with the syringe-dilution method.

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