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THE EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON THE EFFECTIVENESS OF SAMPLERS USED IN AEROBIOLOGICAL STUDIES

By

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SUMMARY

The more promising samplers for the assessment of aerial bacterial populations have been evaluated at 68° F and 65 per cent relative humidity. Even when used with enriched sampler fluid the Detrick all-glass impinger yielded fair to poor recoveries; the TDL slit sampler showed good collection efficiency for high concentrations, but poor for intermediate concentrations of bacterial aerosols; although the Andersen sampler exhibited good collection efficiency, the relative number of viables (colony formers) was disappointing. The best recoveries of viable airborne bacteria were effected with these samplers when the sampling period was two minutes or less.

Studies on the Andersen sampler furnished additional information on the characteristics of solid surface samplers, and also indicated that this sampler possesses a useful degree of size discrimination for bacterial aerosols. Work with the membrane filters shows that the time of exposure of the collected bacterial aerosol to the environment prior to transfer to incubation conditions probably accounts for the low recovery of viables often experienced with this sampler.

It is recommended that future work on samplers be directed toward the development of a more useful and effective sampler for intramural aerobiology.

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I. INTRODUCTION

The specific objective of this project was to determine the effect of various relative humidities and temperatures on effectiveness of samplers commonly employed in aerobiological studies. It had been envisioned that a relatively small amount of additional work would be involved in the use of various samplers during the course of experiments conducted in the prosecution of the objectives of a larger project on the effect of temperature and humidity on airborne bacteria. Also, the results obtained would be of corollary value to the primary researches. Unfortunately, most of the samplers commonly used in aerobiological studies were designed, and are commonly employed for, the assessment of much lower concentrations of bacterial aerosols than we ordinarily employ in our researches. Further, the performance of most of these commonly employed samplers proved to be markedly less effective than our standard laboratory sampler (COLI), and it became apparent that our studies would become more involved in the mechanism of these samplers rather than in the primary study of the effect of temperature and humidity upon airborne bacteria and sampling procedure. In addition, the difference in effectiveness of the various samplers necessitated the use of a large number of replicates to obtain statistically significant quantitative evaluations. For these reasons, towards the end of this project, full-time effort was devoted to the comparative evaluation of those samplers which seemed most likely to us to be useful in future studies. These were the COLI, Andersen, AGI-30 and TDL Slit samplers.

Even though full-time effort during this latter period was expended upon the comparative study of the effectiveness of a few samplers, it was impossible to make comprehensive evaluations because of time limitations. In the light

II. HISTORICAL BACKGROUND

A wide variety of samplers are currently used in aerobiological studies. Characteristics and performance of many of these samplers have been published. However, in most instances, the lack of a uniform and constant source of airborne bacteria make it difficult to evaluate the data presented in the literature.

A. Results of Sampler Studies Obtained by Others

In 1945 dubuy, Hollaender and Lackey (Public Health Reports, Supplement No. 184) published the results of a comprehensive study using the following samplers: air centrifuge, funnel device, atomizer-bubbler device, aeroscope device. Although the ineffectiveness of several of the samplers was indicated, the lack of a standard bacterial aerosol composed of single bacterium particles of uniform size prevented these authors from obtaining exact quantitative data for samplers examined.

Kluyver and Visser (Antoine van Leeuwenhoek, 16, 299-310 (1950)) examined the performance of the Folin bubbler, the Wheeler bubbler, the Moulton atomizer and the capillary impinger. Because bacterial spores were employed in this study, the results are indicative of the efficiency of collection but not of the effectiveness for the demonstration of viable sensitive organisms. Solberg, Shaeffer and Kelly (Ohio J. Sci. 56, 305-13 (1956)) compared the effectiveness of an electrostatic sampler, the funnel device, settling plates and an impinger for the collection of airborne vegetative bacteria. Here, also, the absence of a standard bacterial aersol makes difficult the exact evaluation of their data.

the samples are collected on agar surfaces. The study of a number of factors affecting sampling of bacterial aerosols with the COLI sampler by Cown, Kethley and Fincher (Appl. Microbiol. 5, 119-224 (1956)) demonstrated that, properly designed and used, the critical orifice liquid impinger exhibits a high degree of effectiveness in sampling bacterial aerosols. The chamber systems employed by the Aerobiology Group at Georgia Tech for aerobiological studies meet all the requirements as outlined by Lester in an above reference.

No. 40 atomizer into a prechamber for gravitational settling of gross particles; the resultant aerosol containing essentially single bacterium-beef extract solids particles (Kethley, Fincher and Cown: Appl. Microbiol. 4, 237-243 (1956)). Upon issuing from the prechamber, the aerosol was diluted with air of the desired temperature and humidity and introduced into a 4-ft cubical aerosol chamber (Kethley, Cown and Fincher: Appl. Microbiol. 5, 1-7 (1957)). For the studies reported, only dynamic chamber conditions at 68° F and 65 per cent relative humidity were employed. During operation of the aerosol system, all equipment and materials, including the air samplers, were at the temperature for the condition of the experiment.

B. Media and Bacteriological Procedure

1. Liquid Impinger Media

Unless specifically noted at point of use, the medium used in the liquid impinger samplers consisted of 0.2 per cent gelatin (Pharmagel A) buffered with 0.08 g anhydrous Na_2HPO_4 per g of gelatin and enriched with 1.6 per cent brain heart infusion. Dow AF antifoam was added according to the requirements of the particular sampler and duration of operation.

2. Solid Surface Media

Both nutrient gelatin (128 g/L) and nutrient agar (23 g/L) were used in preparing the Petri plates for solid surface sampling. The gelatin plates were prepared by pipetting 27 ml warm (50° C) sterile gelatin into sterile Petri dishes and placing in a refrigerator (10° C) to firm. Upon solidifying, the plates were inverted and left in the refrigerator for 24 to 48 hours until needed. The agar plates were prepared in a like manner but were stored in the culture laboratory at room temperature (25° C). The storage period of 24

All media were standard Difco preparations (Difco Laboratories). The water used in making serial dilutions, dissolution of solid media plates and preparation of bacteriological media was first passed through a mixed-bed, ion-exchange column (IRA 400 and IR/120, Rohm and Haas). As an added precaution, 1 per cent peptone was added to the dilution water to protect against any trace of chlorine compounds not removed in the above treatment.

C. The Aerobiological Samplers

The samplers employed were selected as being the most likely to be of value in future aerobiological work.

1. COLI

Samplers of this designation were either 1 or 5 L/min critical orifice liquid impingers, 1 quart milk bottles being used as sample holders. Unless otherwise stated at point of use, these samplers contained 200 ml of the enriched fluid, one loop of Dow antifoam being added to each bottle for 1 L/min orifices and 2 to 4 loops for 5 L/min orifices. The bottles containing the fluid and antifoam were plugged with cotton and sterilized. The impinger tips were sterilized and dried separately, the components being assembled just prior to use. Detailed description and use of these samplers have been published (Kethley, Fincher and Cown: Appl. Microbiol. 4, 237-43 (1956) and Cown, Kethley and Fincher: Appl. Microbiol. 5, 119-24 (1956)).

2. Andersen

This sampler more or less combines the principles of both the cascade impactor and seive sampler into a single instrument, consisting of a stacked series of six stages through which the aerosol is drawn. Each stage represents successively smaller sieve holes, there being 400 holes per stage. In

3. AGI-30

This sampler is frequently referred to as the standard all-glass impinger and was manufactured by Scientific Glass Apparatus Company, Incorporated, Silver Springs, Maryland, according to specifications set forth by the Chemical Corps-Camp Detrick M Division in drawing No. C-93-1-3958, as revised 3 April 1957. Flow rates of these samplers were in the 12 L/min range, with one being slightly higher than 15 L/min. In practice, the samplers were operated singly to assure that conditions necessary for critical pressure ratios were maintained for these orifices.

Except where stated, the medium used in these samplers was the same enriched gelatin fluid employed with the COLI samplers, and the sampling period was 5 minutes. For these experiments, 20 ml of the impinger fluid and 4 loops of Dow antifoam were added to the sampler container followed by plugging with cotton and sterilization. The impinger tops embodying the orifice tips were sterilized and dried separately, the units being assembled prior to use as with the COLI samplers. After exposure to the aerosol, proper aliquots of the sampler fluid were plated in the usual manner. Liquid loss occurring during operation of the sampler was determined by either weight or volume measurements.

4. TDL Slit

This sampler was developed by the United States Public Health Service, Communicable Disease Center, Technical Development Laboratory, Savannah, Georgia, and operates in a manner similar to the Bourdillon Slit Sampler (Bourdillon, Lidwell and Lovelock: Studies in Air Hygiene, H.M.S.O., (1948)). The sampled air is drawn through a slit 0.03 in. by 1 in. at 1 cfm, impinging

IV. COMPARATIVE EVALUATION OF SELECTED AEROBIOLOGICAL SAMPLERS

A. General

As pointed out in the introduction, it became impractical to blend the study of the characteristics and merits of various aerobiological samplers into the main effort of the Aerobiology Group. In order to obtain a reasonable evaluation of selected samplers, a short period of intensive study was devoted to those samplers which have shown the most promise in producing both qualitative and quantitative assessment of bacterial aerosol populations. The information necessary for the complete evaluation of these aerial samplers from the standpoint of range of aerial concentration, differential bacterial viability and particle size under various conditions of temperature and relative humidity was not obtained. However, sufficient data were gathered to bring out some of the deficiences of the samplers and to point out some of the requirements of aerial sampling which are not satisfied by any sampler presently available unless the conditions of interest are specifically matched with the characteristics of the particular sampler.

B. Work Procedure and Presentation of Data

The procedure employed in establishing the test aerosol, general bacteriological techniques and handling of the samplers are given in the section of this report devoted to these subjects. Where possible, techniques were standardized for the handling of each sampler and the information in the referred section will apply throughout unless noted otherwise.

It should be emphasized that this work is presented with certain qualifying limitations in that the data apply only for the specific situations reported.

2. Use of Serratia Marcescens and Escherichia coli

There does not appear to be any appreciable difference in the recovery of the two organisms. A difference may have been noticable under other experimental conditions. However, a sampler demonstrating less than 90 per cent recovery efficiency would not likely detect any other than extreme differences.

3. Andersen

This sampler shows good collection efficiency for both high and intermediate concentrations of bacterial aerosols as evidenced through use of the gelatin plate technique. Demonstration of viables (colony formers) where all plates were nutrient agar was of a lower order. The short sampling periods necessary with this sampler require special precautions for higher aerosol concentrations.

4. AGI-30

This sampler yielded fair to poor recoveries even though employed with enriched sampling fluid. Extreme agitation during sampler operation may cause possible re-atomization of previously entrapped organisms during operation.

5. TDL Slit

Though the number of observations were limited, the slit sampler showed good efficiency for high concentrations of bacterial aerosols, but poor efficiency for intermediate concentrations. This latter point was true for both the agar and gelatin plates. As with the Andersen, the time of operation required is very dependant upon aerosol concentration.

TABLE I

COMPARISON OF VARIOUS SAMPLERS WITH CRITICAL ORIFICE, LIQUID IMPINGERS AT 68°F AND 65 PER CENT RELATIVE HUMIDITY, SERRATIA MARCESCENS AEROSOL

Sampler	Number of Experiments	Number of Samples	Average Internal C. V., Per Cent	Average Recovery, Per Cent†
RANGE OF VIABLE ORGANISMS PER LITER CHAMBER AIR: 50-300				
COLI-(5 L/min)	10	79	10.5	100
Andersen-Gel.	8	24	11.8	94
Andersen-N. A.	8	25	16.3	73
TDL-Slit-N. A.	4	15	4.7	70
AGI	8	43	12.6	68
TDL-Slit-Gel.	3	16	13.6	53
RANGE OF VIABLE ORGANISMS PER LITER CHAMBER AIR: 5,000 - 8,000				
COLI-(1&5 L/min.)	4	36	11.1	100
Andersen-Gel.	3	11	8.0	97
TDL-Slit-Gel.	2	9	9.4	95
AGI	2	12	9.1	85

†Recoveries obtained with COLI samplers for each run taken as 100 per cent.

TABLE III

RECOVERY OF AIRBORNE SERRATIA MARCESCENS WITH COLI SAMPLER, 68°F AND 65 PER CENT RH

Run	Number Viables Per L of Air	Sampling Time, Min.	Number of Samples	C. V.	Recovery† Per Cent
A	171	20	9	3.8	100
D	54	20	9	6.5	100
E	85	20	6	5.4	100
F	[illegible]	20	9	6.3	100
G	[illegible]		9	10.3	100
H	[illegible]		8	14.6	100
I	[illegible]		7	12.7	100
J	[illegible]		8	14.2	100
K	[illegible]		7	16.2	100
L	[illegible]		7	15.1	100
B	[illegible]		9	15.7	100
C	[illegible]		9	10.3	100
M	[illegible]		9	8.4	100
N	[illegible]		9	9.9	100

†Res[illegible] each experiment taken as 100 per cent.

TABLE V

RECOVERY OF AIRBORNE SERRATIA MARCESCENS WITH ANDERSEN SAMPLER†, 68°F AND 65 PER CENT RH

Run	Number Viables Per L of Air	Sampling Time, Min.	Number of Samples	C. V.	Recovery, Per Cent††
A	122	1	3	1.5	71
D	28	1	3	43.2	52
F	80	1	3	9.8	111
G	53	1	3	23.3	70
I	94	1	5	8.5	87
J	102	1	3	17.1	79
K	125	1	2	----	46
L	89	1	3	8.2	67

†Nutrient agar plates used in all stages.

††Recovery calculated on basis of setting results obtained with COLI sampler for each experiment equal 100 per cent.

TABLE VI

RECOVERY OF AIRBORNE SERRATIA MARCESCENS WITH TDL SLIT SAMPLER†, 68°F AND 65 PER CENT RH

Run	Number Viables Per L of Air	Sampling Time, Min.	Number of Samples	C. V.	Recovery, Per Cent††
A	91	1/2	5	5.1	53
D	48	1/2	3	2.1	89
E	78	1/2	2	----	92
F	40	1/2	5	7.2	54

†Nutrient agar plates.

††Recovery calculated on basis of setting results obtained with COLI samplers for each experiment equal 100 per cent.

TABLE IX

RECOVERY OF AIRBORNE ESCHERICHIA COLI WITH COLI SAMPLER, 68°F AND 65 PER CENT RH

Run	Number Viables Per L of Air	Sampling Time, Min.	Number of Samples	C. V.	Recovery, Per Cent†
R	85	20	8	9.4	100
S	31	20	9	9.7	100
T	52	20	9	5.5	100
U	34	20	6	6.7	100
V	37	20	9	8.1	100
O	1,710	10	6	7.0	100
P	1,630 (1670)	10	9	10.4	100
Q	7,500	10	9	9.3	100

†Results obtained with COLI samplers for each experiment taken as 100 per cent.

TABLE X

RECOVERY OF AIRBORNE ESCHERICHIA COLI WITH AGI SAMPLER, 68°F AND 65 PER CENT RH

Run	Number Viables Per L of Air	Sampling Time, Min.	Number of Samples	C. V.	Recovery, Per Cent†
R	25	5	6	14.1	30
S	12	5	6	12.9	40
T	22	5	5	14.2	42
U	19	5	6	10.0	56
V	26	5	6	8.8	70
P	1,034	5	6	8.9	62
Q	5,310	5	3	3.7	71

†Recovery calculated on basis of setting results obtained with COLI sampler for each experiment equal 100 per cent.

V. EFFECT OF VARIOUS IMPINGER MEDIA ON COLLECTION AND DEMONSTRATION OF AIRBORNE BACTERIA

Various workers in the field of aerobiology have reported results of their experimentation using liquid impinger type samplers containing media of various compositions. Past experience with the COLI sampler has shown that while the use of any one medium might appear to be quite satisfactory from the standpoint of comparative intra-sampler performance, the collection and demonstration of the maximum number of viable bacteria may not be taking place. Earlier work (Cown, Kethley and Fincher: Appl. Microbiol. 5, 119-224 (1957)) showed that enrichment of the buffered gelatin media with brain heart infusion resulted in a considerable increase in the number of viables demonstrated with the COLI sampler.

On the basis of this past experience, some runs were made with the AGI sampler (10 minute sampling period) employing media of varying composition and comparing these results with those obtained with the COLI sampler for the same conditions. The data in Table XIII show similar improvement with the enriched media.

It has not been shown just exactly whether the increased recovery with the enriched fluid is due to physical, chemical, biological, or a combination of factors. We feel that with the more simple fluids all of the potentially viable cells collected from the bacterial aerosol cloud are not demonstrated due to loss of many of the weak or injured cells within the group of low viability potential because of the absence, in the collecting medium, of suitable constituents (DallaValle, Kethley, Cown and Fincher: Final Report, NIH G-2771 (1956)). Efforts to increase the viscosity by replacement of the gelatin in the enriched gelatin fluid and with more viscous materials and thereby enhance the physical collection did not show any promise.

VI. EFFECT OF SAMPLING TIME ON RECOVERY OF VIABLE AIRBORNE BACTERIA

A. General

The period of time of operation of the various samplers employed in the majority of the work included in this report was of necessity a compromise, so that each sampler could be used in assaying the viable population of the same bacterial aerosol. The working concentrations established in the chamber system ranged from 50 to 5,000 cells per liter of chamber air and the designed rate of flow required for operation of the samplers varied from 1 to 28.9 L/min. The combination of these two factors dictated certain limits of time periods which would yield useful results because the number passing into any given sampler is governed by the time-volume relationship of the sampler and the chamber concentration.

The schedule of this work program did not allow for a thorough investigation of the sampling time factor. However, sufficient information was obtained during the execution of this study to illustrate that variation in the length of the sampling period is often an important factor in considering the assessment of viable airborne bacteria as recovered and demonstrated by a particular sampler. The factors leading to the variation in recovery and demonstration of viable airborne bacteria per unit of operation are undoubtedly dependant upon the individual characteristics of each sampler and conditions of use as well as upon the general character and behavior of the aerosol being sampled.

B. Consideration of Time Factor with Different Samplers

1. COLI

Many of the possible variables affecting efficiency of collection by liquid impinger and bubbler type samplers were discussed in a previous report

especially where agar plates were used in each stage. The scatter of the results obtained in the early use of this sampler made it necessary to provide more accurate control for the short sampling period and to provide a purge system for clearing out any unsettled particles. The data shown in Table XV were collected prior to the installation of the above desired features and indicate the range of variation possible with both high and intermediate aerosol concentrations.

4. TDL Slit

The rotating mechanism of this slit sampler is designed to cover one revolution in 0.5 minute with no provisions for changes. Therefore, no variation from this time was desirable when using agar plates because a second or additional revolutions would have resulted in excessive overlap of deposition. Times varying from 1 through 5 minute were employed with the gelatin plates at the intermediate aerosol concentration for one run and the data showed a coefficient of variation of 21 per cent but no particular trend, due to the scatter of the data.

5. Millipore Filter-AA

Results of varying the time of passage of aerosol through this sampling material is well demonstrated by the results shown in the section of this report devoted to this subject. Although we believe the diminution of demonstrated recovery is due to the length of time the organism is deposited on the surface of the filter prior to addition of favorable nutrients, such diminution should automatically be evident over a prolonged sampling period as the same conditions from standpoint of early deposits would prevail.

VII. TEMPERATURE AND HUMIDITY CHANGES WITHIN THE ANDERSEN SAMPLER

The Andersen sampler having shown considerable promise for aerobiological studies, demonstrating a collection efficiency of at least 90 per cent, a series of studies were carried out to determine the changes occurring within the sampler during operation. It was hoped that such information would be of value in future studies, employing the Andersen plates in experiments on the effect of changes in temperature and humidity on airborne bacteria.

A. Determination of Temperature of Air at Various Stages

To determine the temperature of the air leaving a stage of the sampler during operation a thermocouple of No. 24 gage copper-constantan wire was mounted rigidly within the cavity of the bottom piece of the sampler, isolated from the metal walls of the sampler. An ice-reference junction was employed, and the thermal emf fed into a General Electric Self-Balancing Potentiometer; the output of the potentiometer went to an Esterline-Angus 0-1 milliampere recorder. The system as used was previously calibrated in °F and had a sensitivity of at least 0.5° F, and a response time of less than 1 second (Kethley and Cown: The Research Engineer: 1948-49, No. 5, 5 (1949)).

B. Determination of Dew Point of Air at Various Stages

To determine the dew point of the air leaving a stage of the sampler during operation, a sampling tee was fixed to the exit of the sampler, and 800 cc per minute withdrawn continously through one side of a Gow-Mac Thermal Conductivity Cell. The other side of the cell was constantly flushed with 800 cc per minute of air drawn from a container of activated silica gel. The unbalance in the conductivity cell bridge was fed into a General Electric Self-Balancing Potentiometer; the output of the potentiometer went to an

values were derived from these, employing psychrometric charts. It is to be noted that the relative humidities shown in these figures are higher than those given by Andersen (J. Bacteriol. 76, 471 (1958)); the source of the difference is not known, but we feel that the conditions under which our measurements were made are such that some confidence can be placed in the values shown in Figures 1-5.

Examination of the information presented in Figures 1-5 reveals the fact that, although the temperature and dew point of the air leaving any stage of the Andersen decrease during operation, the relative humidity of this air instantaneously assumes a value which remains constant for the period of time of observation (up to 50 minutes). This is due to evaporative cooling at the surface of each agar plate, the final temperature being influenced by the wet bulb temperature of the air presented to each plate. The actual temperature of the surface of the agar in any stage at any time is almost exactly that of the wet bulb temperature of the entering air, as indicated by the dew point of the air leaving that stage. These values are shown in Table XVI, the values for the dew point temperatures of the air leaving a stage being assumed as the probable values for the temperature of the surface of the agar in each particular stage. It should be noted that the values shown in Table XVI for dew points of the air leaving a stage are sometimes higher than those of the wet bulb temperatures of the entering air for that stage. This is due to the effective heat transfer characteristics of the aluminum of which the sampler is constructed. Direct evidence of this transfer is to be found in the dry bulb temperatures of the output air given in Table XVI, which diminish to 73° F when leaving stage No. 3, remain at this value when leaving stage No. 4, and rise to above 75° F in subsequent stages.

Although there was no opportunity during the span of the present project to capitalize upon the findings presented in this section of this report, it is planned to utilize them in the continuing studies on the effect of temperature and humidity on airborne bacteria.

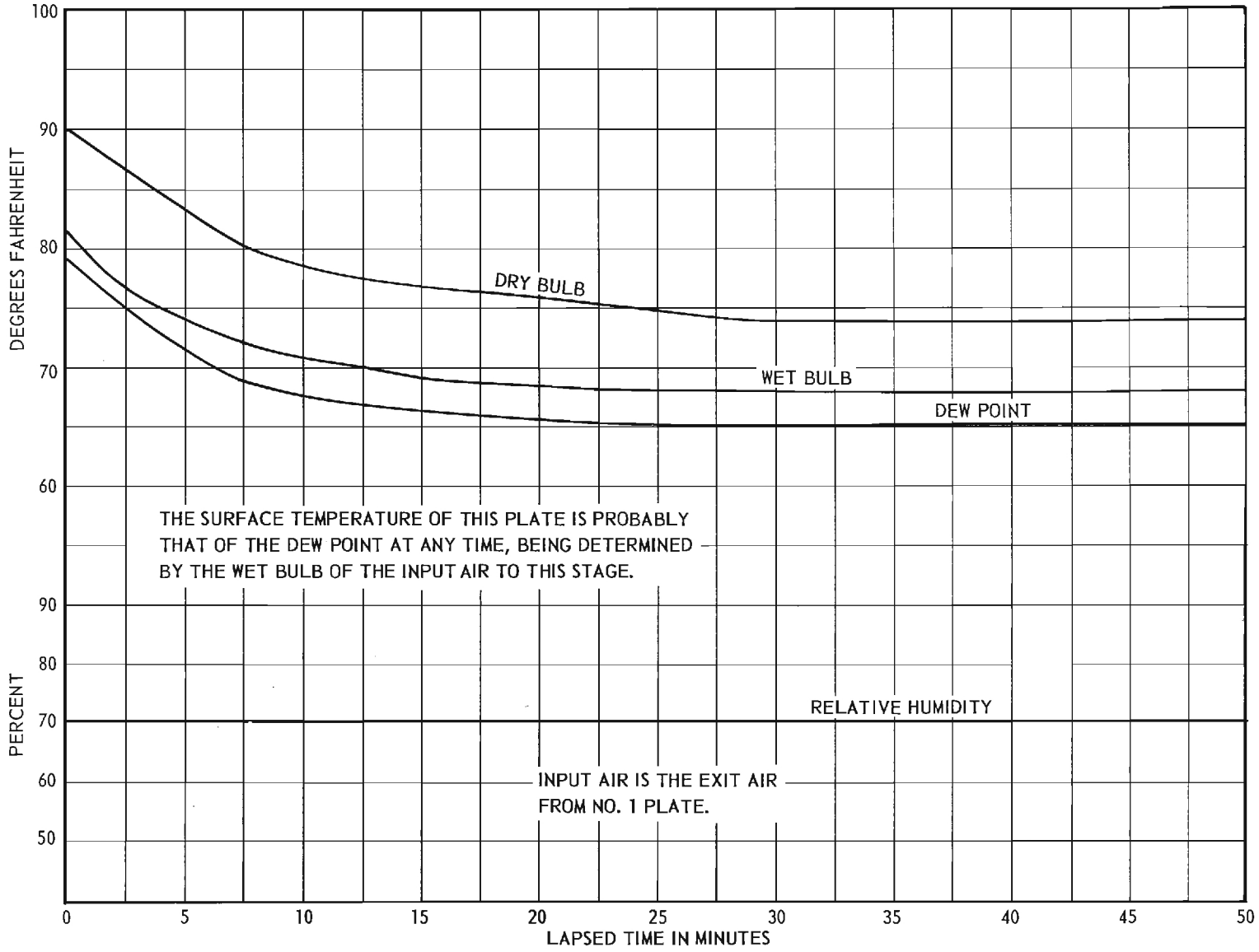

Figure 2. Andersen Sampler, Exit Air from No. 2 Plate.

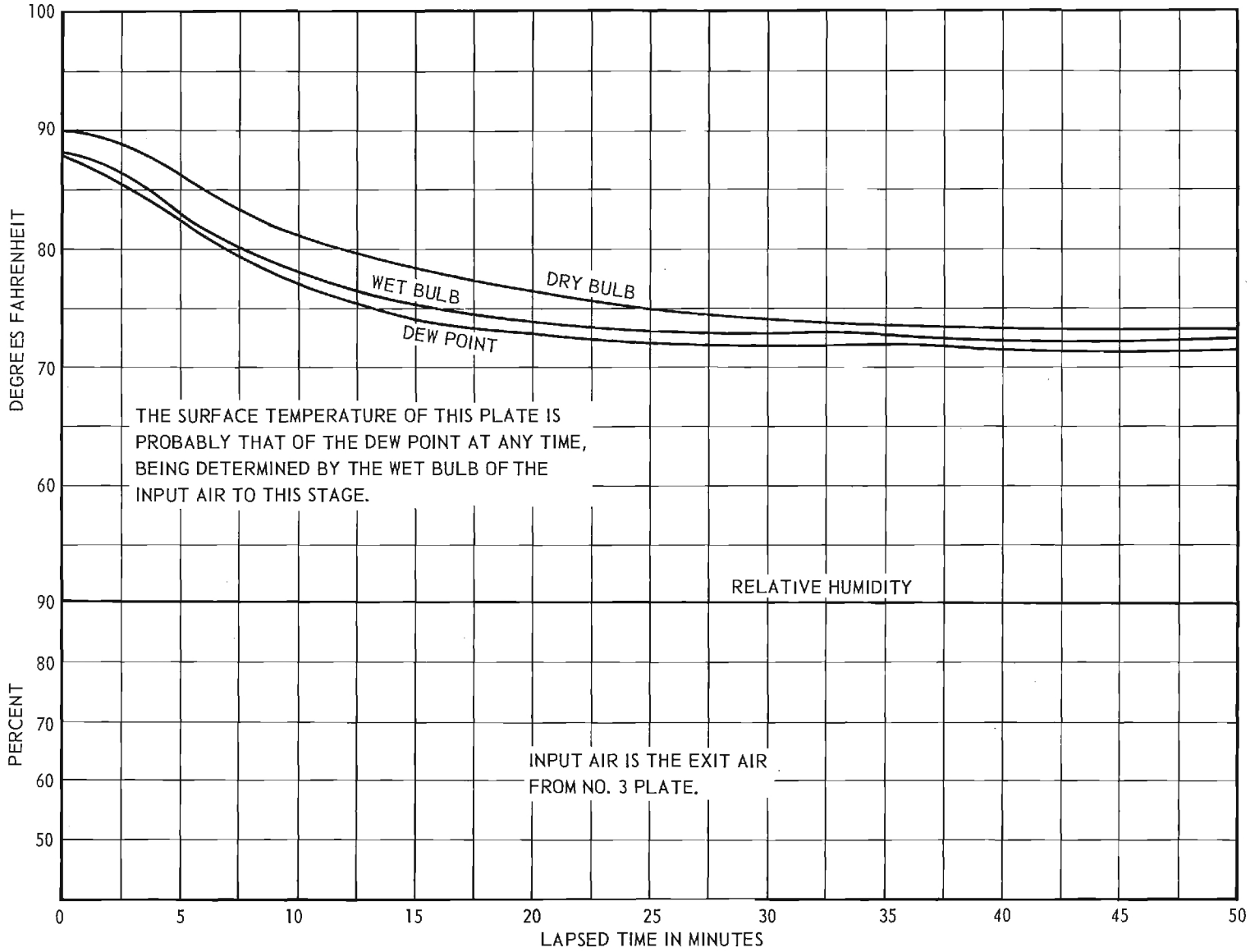

Figure 4. Andersen Sampler, Exit Air from No. 4 Plate.

TABLE XVI

RELATION BETWEEN FINAL WET BULB TEMPERATURES OF INPUT AIR AND FINAL DEW POINT TEMPERATURES OF OUTPUT AIR FOR STAGES OF THE ANDERSEN SAMPLER

	Input Air		Output Air		
Stage No.	Dry Bulb °F	Wet Bulb °F	Dew Point °F	Dry Bulb °F	Per Cent Relative Humidity
1	90	57	58	76	52
2	76	65	65	74	70
3	74	68	68	73	84
4	73	70	72	73	90
5	73	72	73	75	95
6	75	74	75	75+	99

TABLE XVII

PROBABLE RELATIVE HUMIDITIES OF AIR ENTERING THE SEVERAL STAGES OF ANDERSEN SAMPLER FOR VARIOUS HUMIDITIES OF THE SAMPLED AIR

Sampled Air Per Cent Relative Humidity	Per Cent Relative Humidity, Air Entering the Several Stages				
	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
20	55	70	80	85	90
40	60	80	85	90	93
60	75	85	90	93	95
80	85	90	93	95	97

VIII. ANALYSIS OF DATA OBTAINED FROM VARIOUS STAGES OF THE ANDERSEN SAMPLER

A sufficiently large number of samples were taken with the Andersen sampler to warrant analysis in terms of apparent particle sizing effected by the sampler, both in terms of presumed collection efficiency and presumed biological effectiveness (recovery efficiency). Collection efficiency is presumed to be indicated by the results of studies employing gelatin plates, and biological effectiveness from those employing agar plates in the sampler. As previously indicated, the basis for this distinction lies in several factors: the fact that the aerosol utilized is made up almost entirely of single bacterium-carrying particles; the fact that the gelatin plates were removed quickly after sampling and the collected bacteria placed immediately in a known favorable environment; our previous observations that (for reasons as yet undetermined) agar surfaces do not always demonstrate all of the collected bacteria as viables, even when other methods indicate these organisms are capable of colony formation when placed in a favorable (enriched liquid)environment..

A. Particle Size Distribution According to Data of Andersen

According to Andersen (J. Bacteriol. 76, 471 (1958)),for smooth spherical particles of unit density, the various stages of the sampler should collect 95 per cent of particles of the following diameters in microns: stage No. 1, 8.2 and larger; stage No. 2, 5.0 to 10.4; stage No. 3, 3.0 to 6.0; stage No. 4, 2.0 to 3.5; stage No. 5, 1.0 to 2.0; stage No. 6, up to 1.0. However, these were for special particles, and the relative numbers of the smaller particles within the tested aerosol were not indicated.

B. Probable Size Distribution Employing Standard Test Aerosol

A complete analysis of the bacterial aerosol actually employed in these particular studies has not been completed, in terms of size distribution, and

TABLE XIX

PROBABLE HUMIDITIES AND AVERAGE PARTICLE SIZES PRESENTED TO THE SEVERAL STAGES OF THE ANDERSEN SAMPLER, WHEN SAMPLING DILUTED STANDARD BACTERIAL AEROSOL AT 68°F, 65 PER CENT RELATIVE HUMIDITY

Stage No.	Per Cent Relative Humidity of Input Air to Each Stage	Average Particle Size, μ
1	65	< 2.0
2	75	2.0
3	85	2.1
4	90	2.5
5	93	2.6
6	95	2.8

TABLE XX

RELATIVE COLLECTION PER STAGE OF ANDERSEN SAMPLER, SAMPLING BACTERIAL AEROSOLS AT 68°F, 65 PER CENT RELATIVE HUMIDITY

Bacterial Aerosol	Stages	Number of Samples		Cumulative Collection Per Stage as Per Cent of Total			
				Range		Median	
		Gelatin	Agar	Gelatin	Agar	Gelatin	Agar
Serratia Marcescens	1 - 3†		54		0.1-0.5	0.1††	0.1
	1 - 4		49		1.0-14.9	3††	3
	1 - 5	33	16	40-98	40-73	77	67
	1 - 6	33	16			100	100
Escherichia Coli	1 - 3†		31		0.1-0.9	0.1††	0.1
	1 - 4		31		0.7-17.0	3††	3
	1 - 5	21	8	45-98	50-91	85	72
	1 - 6	21	8			100	100

†Without exception, stages 1 and 2 yielded sterile plates, or at most 2-4 colonies which probably were contaminants.

††Gelatin plates were used only in stages 5 and 6 because of the small number collected at any time on plates 1-4.

gelatin plates with those indicated by the agar plates. The overall comparative effectiveness of these methods against the COLI sampler is: over 90 per cent for the gelatin; approximately 70 per cent for the agar. For aerosols of both S. marcescens and E. coli, there appears to be a difference in the numbers demonstrated on stage No. 5, comparing gelatin plates to agar plates. In both instances, the agar plates demonstrating a lower per cent of the total collection on that stage. The difference in comparative effectiveness is presumed to be caused by the failure of some of the collected bacteria to colonize on the agar surface; the difference in per cent of total demonstrated on stage No. 5 suggests that it is at this stage that most of this failure occurs. However, stage No. 5 consistently showed the greatest number of colonies, and if the number of bacteria failing to produce colonies is a random value, then the greatest number of failures would be indicated on the plate having the greatest total number. This assumption could only be verified by the results of experiments employing smaller or larger diameter particles, thus shifting the location of the plate which collects the greatest number of total bacterial particles. It is planned to pursue this in connection with our continuing researches on the effect of temperature and humidity on airborne bacteria.

Although the data presented herein have not been analyzed too critically, such evaluation as has been made indicates the worth of the Andersen sampler in the study of bacterial aerosols. Although the values for size discrimination given by Andersen may not apply to our bacterial aerosols, the sharpness of the cut-off implied by our data demonstrates that this sampler could furnish information adequate for the estimation of the size of bacterial aerosols

IX. MILLIPORE FILTERS

Although membrane filters (Millipore, MF) are efficient collectors of bacterial aerosols, the numbers of viables (as shown by colony formation) are usually disappointingly low. Experiments by others suggested a relationship exists between time of sampling and numbers of viables- the number decreasing with increasing sampling time. A few preliminary experiments in our laboratories indicated a relation between the sampling period and preparation of the MF for incubation and the number of viables. Interpreting these results to mean the total time exposure is important, a single series of experiments was carried out to test this hypothesis. These were carried out in the Aerobiology workroom at 68° F, 70 per cent relative humidity, the standard bacterial aerosol of Serratia marcescens being set up in the 4 ft cubical aerosol chamber. Aerial concentration was determined from samples taken with 1 L/min COLI samplers, employing enriched gelatin fluid. Subsequent to the experiment the net death rate of the airborne bacteria was determined statically in the chamber employing agar plates exposed for 1 minute intervals over a period of 30 minutes. The airborne particles were approximately 2 microns diameter, and the settling data were corrected for mechanical losses.

Fifteen individual samples of the bacterial aerosol from the chamber were taken on MF, each for a period of 2 minutes at 1 L/min, in order to minimize the effect of sampling time. The MF were then suspended in the workroom air, under the same conditions of temperature and humidity as that for sampling, and held for periods of time varying from 50 to 90 minutes. At 10 minute intervals within the time period three MF bearing the sampled

elucidating the effect of temperature and humidity on airborne bacteria because the results would represent the same type of effect that we observe with airborne bacteria. Further, for relatively sensitive bacteria, such as usually found in intramural air, if the method is to yield useful information a prior knowledge of the death rate is required. For resistant microorganisms encountered in extramural air, the method could be applicable. However, the results suggest that the MF might be very useful in the estimation of aerial death rates under those conditions where time lapse cannot be determined by methods such as we employ in chambers or cylinders. For example, in intramural studies, five or more MF samples taken simultaneously, then held for varying periods prior to incubation could serve this purpose. However, neither of these applications are within the main stream of our current investigations and the studies were not pursued.

X. SIGNIFICANT ACCOMPLISHMENTS

Although the efforts directed toward this project did not produce a comprehensive evaluation of a large number of aerial samplers, sufficient quantitative work was accomplished to verify our previous contention that a useful sampler for intramural aerobiological studies must not only be efficient in the collection of bacterial aerosols, but also must present a favorable environment to the relatively sensitive microorganisms normally found in intramural air. On the basis of this contention, we are inclined to doubt that a completely satisfactory sampler exists for field studies of intramural air. A limited comparative study of those samplers considered to be the most useful in aerobiological sutdies was carried out. The results of these studies show that the COLI sampler is highly effective for the study of bacterial aerosol behavior under laboratory conditions but does not meet the requirements dictated by the lower aerosol concentrations encountered in intramural or extramural studies. Of the other samplers examined, the Andersen shows promise of being the most useful because both assessment of numbers and size of the aerial bacterial population may be approximated. Results obtained with MF samples suggest a method for the estimation of aerial death rates under those conditions where other methods could not be applied.