SPACE SCIENCE CENTER

PROGRESS REPORT

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ENVIRONMENTAL MICROBIOLOGY AS RELATED TO PLANETARY QUARANTINE

UNIVERSITY OF MINNESOTA

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Minneapolis, Minnesota

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FOREWORD

From the beginning, the sustaining grant to the Space Science Center has been employed to fund an extensive program of basic studies in environmental microbiology as related to planetary quarantine. The several phases of the interplanetary quarantine investigations have been carried out by staff associated with the School of Public Health under the general supervision of Professor Richard G. Bond, Director of the Division of Environmental Health. Of special interest is the fact that the research activity has provided opportunities for graduate and undergraduate students in public health and related fields to become acquainted with the aerospace program by active participation in research.

In consort with a current phase out of a number of Multidisciplinary space research projects and concentration on a few selected areas, separate reports on each of the major areas will be submitted. Accordingly, this is the first of the major areas of concentration to be reported separately.

The research program as has been described in the previous progress report, has been carried on in several phases; considerable progress has been made in all phases since the last report. Experimentation during this period has been summarized in the enclosed reports.

Warren B. Cheston
Director

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SPACE HARDWARE ASSAY METHODOLOGY

D. Vesley and G. Smith
Department of Environmental Health

INTRODUCTION

A series of experiments has been carried out to help evaluate the N.A.S.A. Standard Procedures for Microbiological Examination of Space Hardware. These procedures constitute an official N.A.S.A document with the purpose of standardizing the methodology of the various laboratories engaged in Planetary Quarantine Research and ultimately for those organizations manufacturing or assembling space hardware subject to Planetary Quarantine restrictions. At the present time an effort is being made to optimize these procedures in terms of maximum recovery of microbial contaminants present, consistent with efficient laboratory procedure. The objective of the following experiments was to determine specifically which of several procedures resulted in greater recovery of viable contaminants under a variety of conditions.

METHODS AND RESULTS

Experiment I

The standard 12 minute ultrasonication of 1" x 2" strips was compared with a 12 minute mechanical shaking process and the use of the standard 1% peptone water eluate was compared with M/15, pH7 phosphate buffer plus Tween 20 as alternative eluates. Comparisons were made on both epoxy strips as well as the standard stainless steel strips and on both hand contact

contaminated strips as well as strips contaminated by aerial fallout.

In the fallout experiments, 80 strips were exposed in an open laboratory for one week. In the handling experiment, 80 strips were handled once by each of four persons. Following handling or fallout, 40 strips were placed in bottles containing 50 ml of 1% peptone solution and 40 in 50 ml of phosphate buffer. Then 20 bottles of each eluate were shaken mechanically and 20 sonicated, both for 12 minutes. Strips were then direct plated in molten TSA. Duplicate 5 ml aliquots of the eluate were removed from each bottle and each mixed with 20 ml of molten TSA. Plates were incubated 72 hours at 32° C. The total count for the two 5 ml aliquots of eluate was multiplied by 5 and added to the direct plate count for a total. Each experiment (stainless steel handled, stainless steel fallout, epoxy handled, and epoxy fallout) was repeated twice, utilizing 80 strips in each experiment, for a total of 640 observations. The results are summarized in Table 1.

TABLE 1
COMPARISON OF METHODS FOR RECOVERY OF VIABLE MICROORGANISMS FROM 1" x 2" STRIPS CONTAMINATED SEVERAL DIFFERENT WAYS

Material	Method of Contamination	Eluent	Mean Colonies/strip (40 strips/condition) after removal by	
			Ultrasonification	Mechanical Shaking
Stainless Steel	Handling	1% Peptone	34.65	17.65
		Phosphate Buffer	103.55	46.85
	Fallout	1% Peptone	37.00	22.75
		Phosphate Buffer	35.00	22.20
Epoxy	Handling	1% Peptone	97.75	66.40
		Phosphate Buffer	98.30	110.75
	Fallout	1% Peptone	32.75	27.25
		Phosphate Buffer	36.30	36.25

Across-the-board comparisons seem to vindicate the choice of sonication over mechanical shaking as a means of dislodging contaminants from strips. On the other hand, phosphate buffer as an eluent appears to yield higher counts than 1% peptone for handled strips, but not necessarily for fallout contaminated strips. Epoxy strips yielded higher counts than the stainless steel. However, the difference appears to be related to hand contact contaminants rather than fallout.

Experiment II

A comparison of removal of microorganisms from fallout contaminated strips in an ultrasonic bath was made to determine the effect of having the contaminated side of the strip facing toward vs. away from the ultrasonic element.

In this experiment, epoxy and stainless steel strips were naturally contaminated by exposure to fallout and assayed by the N. A. S. A. Standards Procedures method. The results are summarized in Table 2.

TABLE 2

EFFECT OF THE POSITION OF CONTAMINATION WITH REFERENCE TO THE ULTRASONIC SOURCE ON THE REMOVAL OF MICROBIAL CONTAMINATION FROM 1" x 2" STRIPS EXPOSED TO AERIAL FALLOUT

Strip Material	Exposed side of strip toward ultrasonic element (80 observations/material)			Exposed side of strip away from ultrasonic element (80 observations/material)		
	Colonies Removed	Colonies Left	Percent Removed	Colonies Removed	Colonies Left	Percent Removed
Stainless Steel	21.50	0.31	98.6	17.00	1.04	94.2
Epoxy	20.80	0.04	99.8	28.70	0.08	99.7
All Strips	21.15	0.17	99.2	22.85	0.56	97.0

When the percent removal is calculated based on the number of colonies remaining on the direct plated strip after elution, the strips facing toward

the sonic element yielded 99.2% of their colonies to assay in the eluate compared to 97.0% for those strips facing away from the element. However, this difference shows up only on the stainless steel strips and not on the epoxy strips.

Experiment III

Stainless steel strips contaminated by natural aerial fallout, natural human handling, and B. globigii spores pipetted onto the strips were evaluated for the following conditions: 1) Comparison of the standard 12 minute sonication with 2 minute sonication. 2) Comparison of the standard 4 ounce bottle with 250 ml flasks in which the strip can be laid horizontally. 3) Comparison of the standard method of placing the container on the floor of the sonicator with suspension of the container from the top of the sonicator.

A total of 96 strips (2 replications of 48) were used in each experiment. These were randomly allocated, 12 strips to each of 8 combinations of conditions, as follows: 1) Bottle - 12 minute sonication - bottom of sonicator; 2) Bottle - 12 minute sonication - top of sonicator; 3) Bottle - 2 minute sonication - bottom of sonicator; 4) Bottle - 2 minute sonication - top of sonicator; 5) Flask - 12 minute sonication - bottom of sonicator; 6) Flask - 12 minute sonication - top of sonicator; 7) Flask - 2 minute sonication - bottom of sonicator; 8) Flask - 2 minute sonication - top of sonicator.

The analysis followed the standard procedure except for variations noted and that no anaerobic incubation was carried out. For the B. globigii experiments, distilled water suspensions from spores stored in ethanol were prepared and 0.1 ml of an approximate 10^3 ml dilution was pipetted onto each strip and spread with a glass rod. Analyses were done about 18 hours after contamination.

The results of these experiments are summarized in Table 3 as ratios of the results for the current standard method to those for the modified technique. The results indicate that regardless of contamination method, better results were obtained with the flask than with the bottle and by the current

standard bottom position as opposed to the top position. The fact that sonication time does not materially influence the result in the range tested indicates that the shorter (2 minute) period could be used to expedite the analysis.

TABLE 3
COMPARISON OF STANDARD METHOD TECHNIQUES
WITH THE MODIFIED TECHNIQUE

Factor	Contamination Method	Ratio of Standard Method to Modified Technique	
		col/strip	% Removal
4 ounce bottle* vs. 250 ml flask	Handling	0.51	0.74
	Aerial Fallout	0.89	0.99
	B. globigii	0.59	0.51
	All	0.66	0.75
12 minute sonication* vs. 2 minute sonication	Handling	1.08	1.01
	Aerial Fallout	0.98	1.00
	B. globigii	0.99	1.15
	All	1.02	1.05
Bottom of sonicator* vs. top of sonicator	Handling	1.17	1.16
	Aerial Fallout	1.21	1.00
	B. globigii	1.28	1.38
	All	1.22	1.18

* Current Standard Technique

FUTURE WORK

Following expected revision of the Standard Procedures in early 1968, it is expected that additional evaluations of similar nature will be carried out.

DIE-OFF OF MICROBIAL CONTAMINATION

D. Vesley and G. Smith
Department of Environmental Health

INTRODUCTION

A series of experiments has been initiated and partially completed concerning the effects of various temperature and O_2 pressure combinations on the die-away of viable contaminants on stainless steel and epoxy strips over an extended time period. An additional condition which has been evaluated has been a laminar downflow room where the air motion effect has been evaluated at ambient temperature and O_2 levels. The purpose of these investigations is to provide basic information on viable count reduction which may be expected on space hardware over given time periods and under given storage conditions.

METHODS AND RESULTS

EXPERIMENT I

To determine the effect of storage in a laminar downflow facility on the die-away over a 12 week period of fallout contaminants on stainless steel and epoxy strips, the N.A.S.A. Standard Procedure for assay of fallout contamination on 1" x 2" strips was used.

Results are summarized in Table 1. The relatively light initial contamination on the stainless steel strips before being placed in the laminar flow room makes analysis difficult. There is no doubt that aerobic nonspore forming organisms do die off under laminar flow conditions. The steady

TABLE 1

DIE-OFF OF NATURAL FALLOUT CONTAMINATION ON STAINLESS STEEL AND EPOXY STRIPS DURING 12 WEEKS OF STORAGE IN A LAMINAR DOWNFLOW ROOM

Time in weeks	Mean Col./Strip (10 Strips/Material/Time Period)									
	Stainless steel strips					Epoxy strips				
	non-heat shocked		heat shocked		total	non-heat shocked		heat shocked		total
	Aerobic	Anaerobic	Aerobic	Anaerobic		Aerobic	Anaerobic	Aerobic	Anaerobic	
0	23.4	1.5	2.0	0.5	27.4	96.4	32.0	36.0	12.5	176.9
1	13.0	0.6	1.1	0.6	15.2	31.2	20.0	32.5	12.5	96.2
2	4.0	0.5	0.5	0.0	5.0	82.2	88.3	33.3	7.8	211.7
4	1.5	0.5	1.5	0.0	3.5	66.0	16.0	29.0	8.0	119.0
8	-	-	-	-	-	21.2	14.5	29.0	9.0	73.7
12	0.0	0.0	0.0	0.0	0.0	51.8	7.0	30.5	5.5	94.8

downward progression from 23.4 colonies/strip at zero time to no colonies after 12 weeks bears this out. However, the organisms resistant to the heat shock procedure and the non-heat shocked anaerobes were so few even at zero time that no significance can be attached to the zero counts obtained after 12 weeks. In an effort to overcome the low initial contamination levels, for experimental purposes, the epoxy strips were exposed in a room housing laboratory animals rather than in a general laboratory area. It appears that the die-off was relatively rapid on the stainless steel strips and much less so on the epoxy. However, analysis of the heat shocked aliquots from the epoxy strips indicates that the level of heat resistant organisms remained quite stable through the 12 weeks. The rapid die-off noted on the stainless steel compared to the epoxy is most likely a result of the very low level of spore formers at zero time on the stainless steel plates. For total count it appears that the die-away on epoxy strips in the laminar flow facility takes place more gradually than it does on stainless steel strips. This tends to bear out previous conclusions. It also appears that the die-away of heat resistant organisms (spores) is much less than it is for those species readily destroyed by heat. This point has been somewhat inconclusive in previous reports concerning the "plateau" phenomenon.

EXPERIMENT II

To further study die-away, a second experiment involving five different storage conditions and four different groups of contaminated strips was carried out over a 28 week period. The five storage conditions were as follows: 1) Ambient room conditions (covered containers at 22° C. and 30-50% RH); 2) 22° C. in anaerobic jars (low O_2, low moisture); 3) 4° C. (in covered containers with no reduction in moisture or oxygen tension); 4) 4° C. in anaerobic jars (low O_2, low moisture); and 5) Laminar downflow room exposed (22° C. and 40-45% RH). The four different groups of strips-contamination were: 1) Stainless steel, fallout; 2) Stainless steel, hand contact; 3) Epoxy, fallout; and 4) Epoxy, hand contact.

Data from these experiments were subjected to an analysis utilizing logarithmic functions in an effort to overcome the "outrider" effect of very high counts which may appear at random at various time periods and various storage conditions. These studies resulted in die-off rates based on the slope of a regression line for each storage condition and each type of analysis (heat shocked, aerobic and anaerobic and non-heat shocked, aerobic and anaerobic). N.A.S.A. Standard Procedures for strip assay were used.

Two of the four experiments have been completed on die-off of naturally deposited contaminants under the five different storage conditions. The results of these two experiments are summarized in Tables 2 and 3. In the experiments very low initial contamination levels were encountered (11.5 colonies/strip for epoxy and 15.5 colonies/strip for stainless steel). In addition, zero time heat shocked aliquots disclosed almost zero counts (0.0 for epoxy and 0.8 for stainless steel). The fact that such low colony counts were obtained even in the absence of sterile gloves or aseptic handling practices is, of itself, of interest. The calculated weekly die-offs of from 1.7 - 12.2% total colonies occur under the various storage conditions, with ambient temperature (about 22° C.) and low O_2 producing the greatest estimated weekly die-off effect (8.5% on stainless steel and 11.1% on epoxy). The inherent high variability makes several of the die-off rates insignificant at the $\alpha = .05$ level. When only the heat shocked portions are considered, the very low initial levels preclude any realistic appraisal of die-off; the highest indicated rate was only 1.3% (on epoxy strips stored at 22° C. and low O_2), and none of the rates from the heat shocked portions is significantly greater than zero.

Preliminary results of the aerial fallout onto stainless steel strips study are interesting to the point that there was no apparent significant reduction except for those exposed in the laminar flow room where a 12.1% weekly die-off was noted for total colonies and 7.0% for aerobically incubated heat shocked aliquots. Thus, there is an indication that the air movement associated with laminar flow may accelerate colony count reduction compared with

TABLE 2

THE EFFECT OF VARIOUS STORAGE CONDITIONS ON THE DIE-OFF OF ORGANISMS OVER A 28 WEEK PERIOD FROM STAINLESS STEEL STRIPS CONTAMINATED FROM NATURAL HUMAN CONTACT

	Storage Condition	Non-Heat Shocked		Heat Shocked		Total
		Aerobic Inc.	Anaerobic Inc.	Aerobic Inc.	Anaerobic Inc.	
Col./strip at zero time*	All	12.1	2.5	0.0	0.8	15.5
% Reduction per week**	Ambient temperature and O_2	3.1	0.8	0.2	0.2	3.5***
	Ambient temperature low O_2	8.3***	5.1	0.1	0.3	8.5***
	4^{o} C., ambient O_2	0.7	0.3	0.8	0.3	1.7
	4^{o}C., low O_2	12.2***	7.0***	None	0.7	12.2***
	Laminar Downflow Room	2.3***	0.5	0.1	0.3	2.4***

* The same data for zero time were used for all storage conditions. Therefore, the estimates for the several storage conditions are correlated.

** Based on computer analysis of data from N.A.S.A. Standard Procedures for strip analysis from 6 strips/condition/time period examined at 0, 1, 2, 4, 9, 12, 17, 20, 24, and 28 weeks.

*** Significantly greater than 0 at $\alpha = .05$.

TABLE 3

THE EFFECT OF VARIOUS STORAGE CONDITIONS ON THE DIE-OFF OF ORGANISMS OVER A 28 WEEK PERIOD FROM EPOXY STRIPS CONTAMINATED BY NATURAL HUMAN CONTACT

	Storage Condition	Non-Heat Shocked		Heat Shocked		Total
		Aerobic Inc.	Anaerobic Inc.	Aerobic Inc.	Anaerobic Inc.	
Col./strip at zero time*	All	6.5	5.0	0.0	0.0	11.5
% Reduction per week**	Ambient temperature and O_2	8.1***	1.6***	0.3	0.4	8.4***
	Ambient temperature low O_2	11.5***	4.6***	1.3***	1.2	11.1***
	4^{o} C., ambient O_2	5.0***	0.3	1.2	0.1	5.6***
	4^{o} C., low O_2	4.6	5.0	None	None	4.3
	Laminar Downflow Room	6.8	4.3	1.0***	None	7.9

* The same data for zero time were used for all storage conditions. Therefore, the estimates for the several storage conditions are correlated.

** Based on computer analysis of data from N.A.S.A. Standard Procedure for strip analysis from 6 strips/condition/time period examined at 0, 1, 2, 4, 9, 12, 17, 20, 24, and 28 weeks.

*** Significantly greater than zero at $\alpha = .05$.

similar temperature and humidity conditions without the air movement.

FUTURE WORK

The experiments currently in progress will be completed in the near future. It is then anticipated that a new series of experiments will be initiated to evaluate die-off under various temperature and relative humidity combinations for model spore systems applied by several methods and previously subjected to a dry heat stress.

METHODOLOGY OF MEASURING INTERNAL CONTAMINATION IN SPACECRAFT HARDWARE

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Department of Environmental Health

INTRODUCTION

Accurate and reproducible techniques for measuring the viable microbial contamination of spacecraft hardware are essential to the success of the planetary quarantine program as it is presently envisioned. In order to prescribe any realistic sterilization treatment, it is necessary to know, among other things, both the total contamination load and the destruction kinetics for the most resistant organisms under the given environmental conditions. The validity of either of these estimates can be no more reliable than the accuracy of the microbiological assay techniques employed in their determination.

To provide answers to the above problems the following specific areas were investigated: (1) What are the most appropriate methods for recovering inoculated spores from a number of different solids? What kind of reproducibility and precision can be obtained with these methods?; (2) What are the effects of surface sterilization treatments on occluded contamination?; (3) How long do occluded contaminants survive under different storage conditions?

RESULTS

The results of this study can be summarized by a number of general statements regarding the assessment of viable microbial contamination found in the interior of spacecraft hardware:

1. Estimates of sterilization effectiveness will depend on knowledge of

the total contamination load and the destruction rates of embedded organisms. This knowledge, in turn, will depend on the accuracy and reliability of the assay technique employed.

2. The accuracy of assay techniques for interior contamination are functions of a) the efficacy of the method used to liberate embedded organisms, and b) the efficacy of the culturing method used. Since these two functions are usually confounded, the best approach toward measuring the efficacy of any given technique is an empirical one, using controlled artificial inocula and model solid systems.
3. No simple assay method is suitable for measuring the interior contamination of all potentially contaminated materials. Where possible, dissolving in a nontoxic solvent is the preferred technique for liberating embedded organisms. Failing this, some methods of nonlethal fine grinding or pulverization must be employed.
4. Interior contaminants may be subdivided into three general categories, each endowed with its own problems of assay, sterilization and potential hazard to planetary quarantine:
   a. Internal contamination of piece parts.
   b. Occluded contamination in potting compounds, adhesives, and encapsulating plastics.
   c. Inaccessible contamination on intimately mated surfaces.

CONCLUSIONS

The research program upon which this report is based concerned itself almost exclusively with assay problems of the second category listed above: Occluded contaminants in polymerizable plastics. The following general and specific conclusions were reached on the basis of the experimental data presented in this report:

1. The accuracy and reliability of any estimate of viable contamination

in the plastics studied is a function of:

a. The specific material in which the organisms are embedded.
b. The method used to liberate the viable spores.
c. The nature of the inoculum used, and in certain cases, the actual concentration of viable spores in the inoculum.
d. The elapsed time between contamination and assay, and the temperature at which the material was stored.
e. Interactions of the above.

2. Simple shattering of contaminated plastics containing $> 10^4$ spores/gm consistently yielded detectable viable spores in the air and on surfaces in the immediate vicinity.
3. Culturing of liberated spores did not present any real difficulties. Trypticase Soy Agar supplemented with Calcium dipicolinate yielded suitable recoveries. There was no real advantage gained by the use of membrane filters (compared to standard pour plates) or freshly poured "molten" agar (compared to prepoured hardened agar). The precision of the culturing data, using spores liberated from plastics, was as good as that experienced in classical bacteriological quantitation.
4. Different organic solvents exert different toxic effects on spores. Acetone, benzene, and ether were not deleterious.
5. Soluble polymer systems, such as paraffin, paraplast, and Rigidax, are useful models whereby to study recovery and pulverization methodology. Water soluble Rigidax was an excellent simulant for hard polymers and permitted recovery of ~ 100% of the original inoculum.
6. Pulverization in a Pica Blender mill for up to two minutes did not exert any deleterious effect on spore viability. Longer exposures were sporicidal. Briefer exposures were inadequate for epoxy size reduction.

7. Polymerized epoxy, containing 10^4 - 10^6 spores/gm, consistently yielded approximately 80% of its theoretical spore load after a preliminary cryogenic disintegration with mortar and pestle followed by two minutes of pulverization with a Pica Blender. Lower yields (40 - 50%) were obtained when the original inoculum was ~ 10^2/gm.
8. Silicone rubber, containing 10^5 - 10^6 spores/gm, yielded approximately 7% of its theoretical spore load after cryogenic sanding and pulverization.
9. Lucite, containing 10^3 - 10^7 spores/gm yielded approximately 74% of its theoretical spore load after dissolving in acetone.
10. The best methods developed and studied for spore recovery from epoxy, silicone rubber, and Lucite provided consistent and reproducible results.
11. Embedded spores survived best under near freezing conditions and demonstrated significant and consistent diminution of viability (and /or recovery) at warmer temperatures.
12. Dry heat at 135 C required more than seven hours to lower the embedded spore concentration in epoxy by one logarithmic cycle.
13. Ethylene oxide did not penetrate beyond the surface of hardened epoxy. However, it did penetrate into the interior of other plastics and exerted some sporicidal effects in Rigidax and paraplast, depending on density and degree of polymerization.

FUTURE WORK

This subproject has been completed and new work while it may continue in the same general direction will have new and specific objectives.

PUBLICATION

Greene, V. W., Walker, B., and Anderson, O. H., "Methodology of Measuring Internal Contamination in Spacecraft Hardware." Final Report under NGR-24-005-063 (Project #1, Phase #2) from National Aeron. Space Admin. June, 1967. pp. 59. 17 Ref.

MEASUREMENT AND CONTROL OF MICROBIAL CONTAMINANTS IN AIR

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INTRODUCTION

Production of a sterile lander for Mars or Venus exploration requires a sterilization cycle which is in turn influenced by the nature and quantity of the microbial burden at the time of sterilization. One source of the microbial burden on the spacecraft is the fallout from the air during construction and assembly of the spacecraft. Control of fallout contaminants may be critical to the construction of sterilizable spacecrafts; however, before we can control microbial fallout from air we must be able to accurately measure this contamination. In this project the measurement of microbial contamination has been studied from several different points which include evaluation of sampling equipment and levels of microbial contamination in different types of air systems.

RESULTS

Extensive tests were carried out which compared the High Volume Casella Sampler (1000 lpm - direct impaction) to the Lundgren High Volume Sampler (1000 lpm - electrostatic) in environments with graded degrees of natural contamination. The following factors were investigated: (1) The optimal collecting fluids for the Electrostatic sampler; (2) The relative efficacy of each sampler in normal environments and in laminar flow clean rooms; (3) The practicality of using the Lundgren device as a sequential sampler for the long time periods; (4) The relative efficacy of both high

volume samplers as compared to classical low volume samplers.

CONCLUSIONS

Conclusions reached from these tests were:

1) The optimal collection fluid for the Lundgren sampler is Trypticase Soy Broth supplemented with 0.1% Tween 80.
2) Since this fluid contains nutrients, any long term sampling (2 hours) with the Lundgren apparatus may involve growth; therefore, for extended sampling periods, the collection fluid must be dispensed and stored at refrigerator temperatures.
3) There is general agreement between the Lundgren device and high volume Casella with regard to measuring airborne viable contamination. The former actually yields somewhat higher counts, but this may be an artifact resulting from break-up of clumps.
4) The Casella High Volume Sampler is simple to operate, is relatively inexpensive, and collects directly on a nutrient surface suitable for incubation and counting. On the other hand, its versatility is limited to contamination levels which yield countable plates (i.e., max = 100/ft.^{3}; min = 0.2/ft.^{3}); furthermore, the brief sampling period (5 minutes) does not permit sequential study of contamination rise and fall unless an operator is present to change plates frequently.
5) The Lundgren High Volume Sampler is expensive, is more complicated and difficult to operate, and requires laboratory manipulation of sample after collection. On the other hand, it can be used to sample any airborne concentration of microorganisms because the sample can either be concentrated or diluted after

collection. Similarly, it can be equipped with a refrigerated fractionator to permit unattended sequence sampling.

6) With regard to clean room sampling, neither of the high volume devices showed any advantage over the Reynier sampler, currently considered as the standard, except that the same information was obtained much more quickly.

A pilot research study was undertaken to gather data regarding the types and numbers of microorganisms distributed along a five-hundred foot vertical profile in the center of a populated, industrially-oriented urban area. It was hoped that this study would provide some baseline information about the microbiology of urban environments, and demonstrate the feasibility of sampling under these conditions, along with indicating the pitfalls that would be encountered.

It appears that there is a gradual diminution in count related to increase in altitude, but the counts, even at the highest level, are still remarkably high. This was our first insight into the reality of turbulent air masses at 500 feet, since contaminated particles, even those as small as 5 in diameter, would settle at 5 to 6 feet per hour. Unless there were a generating source above the tower, in still air there should be a significant difference between the lowest and the highest altitude. These values can be placed in their proper perspective when one recognizes that intramural air in occupied rooms rarely contains more than 50 microorganisms per cubic foot.

FUTURE WORK

Microbial distribution data gathered in the tower study will be correlated with metrological data for the same tower. The results of this analysis will be used to determine the future direction of this project.

DETECTION OF LOW LEVELS OF MICROBIAL CONTAMINATION ON SURFACES BY CHEMICAL APPROACHES

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INTRODUCTION

This is a project to develop chemical methods for rapid detection of small quantities of living organisms, as well as small numbers of "dead" cells and for distinguishing between living cells and the biochemical residue of "dead" cells.

The chemical approach is focused on nucleotides and their derivatives as the most important compounds present in all living things. The experimental work will be divided into three phases:

1) Development of chemical methods for separation and identification of submicro quantities. This will require experimentation to increase the sensitivity of existing chemical micro methods and the development of new ones.
2) Relatively "large" quantities of "living" cells will be hydrolyzed, and the hydrolysate concentrated, separated and derivatives identified with the aid of infra red spectroscopy.
3) Microbial cells treated with sterilizing materials, will be analyzed with methods developed in the first phase. The bacterial cells will be classified as "dead" or "live" according to the degree of degradation of the nucleotide tracer.

In this study, the latest developments in analytical microchemistry and thin-layer chromatography will be used along with infra red spectroscopy and high-voltage electrophoresis. Thin-layer chromatography provides the

capability of separating small quantities of material with high resolution in a very short time--less than one hour.

RESULTS

Initial efforts were spent in a careful search of the literature in the Library of Congress. Several technical reports of NASA projects were read for background information on the chemical and physical conditions to which bacteria on the testing surfaces have been subjected.

The following summary can be made of the literature reviewed:

1) Very little is known about the chemical composition of viable or nonviable microbial cells. Several studies are underway using physicochemical methods based on bioluminescence and enzyme activity.
2) Sterilizing materials (gases) used for space craft and surface bacterial decontamination can contaminate the surfaces chemically. As an example, the impurities in ethylene oxide (ETO) may form sediments on testing surfaces. These sediments collected with the testing material can become interfering substances in submicro chemical reactions.
3) For chemical work it is important to know the structure and the chemical composition of the surface material tested. There is always the possibility that small particles from surface material will be included in the collected micro samples. These particles can act as catalysts in chemical reactions.

Laboratory work is in progress on the study of chemical properties of nucleotides on thin-layer chromatographic plates. In this work adsorbents being tested include: silica gel G, aluminum oxide, cellulose and ion exchange resins. The pH of the suspension of the stationary phase before the application, the particle size of the stationary phase and the influence of the thickness

of the layer are being studied. Combinations of solvent systems for the mobile phase are also under investigation.

FUTURE WORK

The immediate goal in this project is to explore and improve existing methods for the chemical separation and identification of nucleotides. Once this has been accomplished the study will follow generally the steps outlined in the introduction. All of the chemical work will be performed in a clean room where the surfaces to be tested for bacteria will be placed. It is planned that the device for collecting bacterial samples will be a part of the equipment used in the chemical procedure. Shortly before use the device will be sterilized to control outside contamination.

STUDY OF ATTRIBUTES OF MATED SURFACES THAT AFFECT THE HEAT DESTRUCTION OF MICROORGANISMS LOCATED IN THESE AREAS

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Department of Environmental Health

INTRODUCTION

During the past year the scientific community has accepted the idea that microbial spores are destroyed more rapidly during heating when they are located on exposed surfaces than when they are in some type of closed environment either enclosed in a hermetic container or encapsulated in a solid. In spacecraft and landers, microbial contamination not only will be located on surfaces and embedded in solids, but will also be located in mated surfaces.

The present project is an extension of research performed by Angelotti (1967) which indicated that the dry heat D-value of spores in mated surface areas varies with the degree of tightness of the surfaces. The effect is thought to be due to a variation in diffusion of water vapor from the spores. In this project we plan to extend the work by varying the distance of the organisms from an opening, and varying the amount of pressure between the mated surfaces. In this study we plan to investigate the relationship of the heat destruction rate of bacterial spores and: (1) Diffusion distance (or degree of enclosure); (2) Conditioning environment of the spores; (3) Bacterial species. The measurement of heat destruction rates shall include determination of D and Z values.

RESULTS

This project was started near the end of 1967; therefore progress is limited to developing a plan of attack and specific equipment.

A heat block unit has been designed to be used to investigate these relationships. It consists of two 10-inch square blocks with facing plane surfaces and provision for springs to hold the faces together with known pressure. Two 150-watt cartridge heaters are used to heat each block; the heaters are controlled to an accuracy of approximately $+1^{\circ}F$ by a Honeywell R7079A thermistor controller. Test samples will be clamped between the two blocks. The samples in the initial test series will be deposited on stainless steel shim stock (.015 thickness). A cover of the same material will be used and a separator of thin shim stock is optional. The test sample will be packaged in aluminum foil to prevent contamination and to stabilize the conditions inside it.

FUTURE WORK

During the next year we plan to evaluate the effect of the distance from spores to open surface, and thickness of open path on heat destruction rates. Later we hope to relate heat destruction rates with molecular diffusion rates of essential chemicals from the spores.

PRESENTATION

Pflug, I. J., "Dry Heat Sterilization: Rates of Destruction and Temperature Coefficients." COSPAR Symposium on Sterilization Techniques for Instruments and Materials as Applied to Space Research. London, July 18-22, 1967.