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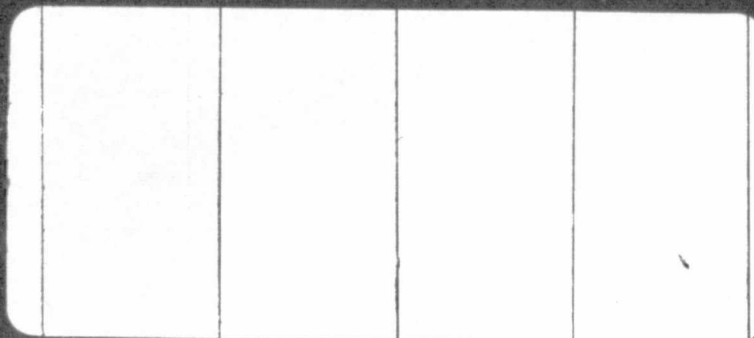
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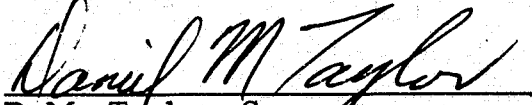
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PLANETARY QUARANTINE

.Semi-Annual Review
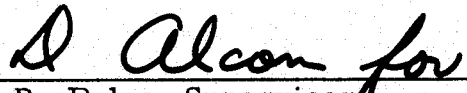Supporting Research and Technology

1 July - 30 December 1975

April 18, 1976

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PREFACE

This document contains a report on Research and Advanced
Development at the Jet Propulsion Laboratory during the
period 1 July 1975 to 30 December 1975, sponsored by the
Planetary Quarantine branch of the NASA Office of Space
Sciences and Applications

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SECTION I

PLANETARY QUARANTINE STRATEGIES
FOR
ADVANCED MISSIONS
(NASA NO. 193-58-61-01)

Contents	Title and Related Personnel
para. 1.1	STRATEGIES FOR SATELLITE ENCOUNTER

Cognizance: C. Gonzalez

Associate
Personnel: W. Brady

1.1   STRATEGIES FOR SATELLITE ENCOUNTER

1.1.1   Introduction

The overall objective of this task is to identify those areas of future missions which will be impacted by planetary quarantine (PQ) constraints. The objective of that phase being described here was to develop an approach for using decision theory in performing a PQ analysis for a Mariner Jupiter Uranus (MJU) Mission and to compare it with the traditional approach used for other missions.

1.1.2   Significant Accomplishments

The MJU Mission is an attractive one for a demonstration of the decision theory approach to PQ analysis. The mission is complex because there are a number of options available at each planet encounter and the option selected at one planet encounter influences subsequent encounters. In addition, so little is known about Uranus that the spacecraft scientific instruments should yield substantially improved information about it well before encounter.

In the analysis done for this study, a number of simplifying assumptions will be made. Since this is a new approach to solving this problem, it was prudent to keep the problem simple. Reducing the complexity of a model to the essentials allowed it to be exercised, while an understanding of the important features was gained prior to consideration of a complete model.

The approach advocated in the reference for performing quarantine analysis, can be explained most easily by a simple example. Consider a spacecraft which is sent to a distant planet about which very little is known. Assume also that the spacecraft is carrying scientific instruments which are capable of supplying the information necessary to improve the estimates of the probability of growth of terrestrial organisms by a substantial margin. Not only can these instruments make these valuable measurements, but they can make them weeks before encounter. Knowing this, scientists could hypothesize various possible outcomes of this experiment and estimate probability of growth for each outcome.

For simplicity, two probabilities of growth were designated, low and high. The scientific yield that is possible will be inversely proportional to the

probability of growth because the PQ is less restrictive when the probability of growth is small. For each level of probability of growth, there is a tradeoff between the risk of contaminating the planet and the science value.

In order to maximize the mission value, two maneuvers were considered; it was assumed that the first maneuver was successful. The science value and the penalty associated with contaminating the planet (a negative value) were placed on the same value scale and the value associated with each possible mission outcome was evaluated.

Figure 1-1 shows a simplified value tree developed for the example mission. Once all of the values of the outcomes are known and the probabilities of the outcomes are known, the value of each decision at each decision node can be evaluated and the optimum decisions can be determined. Once the solution is complete, a probability of contamination can be computed.

There are two classes of decisions to be considered in such an analysis. One class consists of those decisions that are made prior to the mission regarding what should be done for each possible mission outcome. For example, the Jupiter encounter conditions will be influenced by the requirement to continue to Uranus, the Jupiter science, the health of the spacecraft, etc. If it is assumed that the spacecraft is in perfect condition, there will be some aimpoint that is considered to be optimum for that situation but which would not be appropriate if the imaging system has failed. Assuming a healthy spacecraft, the selection of this aimpoint can be made prior to launch. The remaining decisions are those which must be performed during the mission. If the imaging system fails, the accuracy of the orbit determination is degraded and a different aimpoint may be appropriate.

Figure 1-2 shows an example of a decision tree for the last maneuver prior to Jupiter encounter. The designations G_1, G_2, and G_3 are used to describe the condition of the guidance system. The definitions are given below:

Symbol	Definition	Probability
G_1	Optical and radio systems functions	0.99
G_2	Only radio system functions	0.01
G_3	No guidance system available	1×10^{-5}

Fig. 1-1. Structure of the Basic Mission
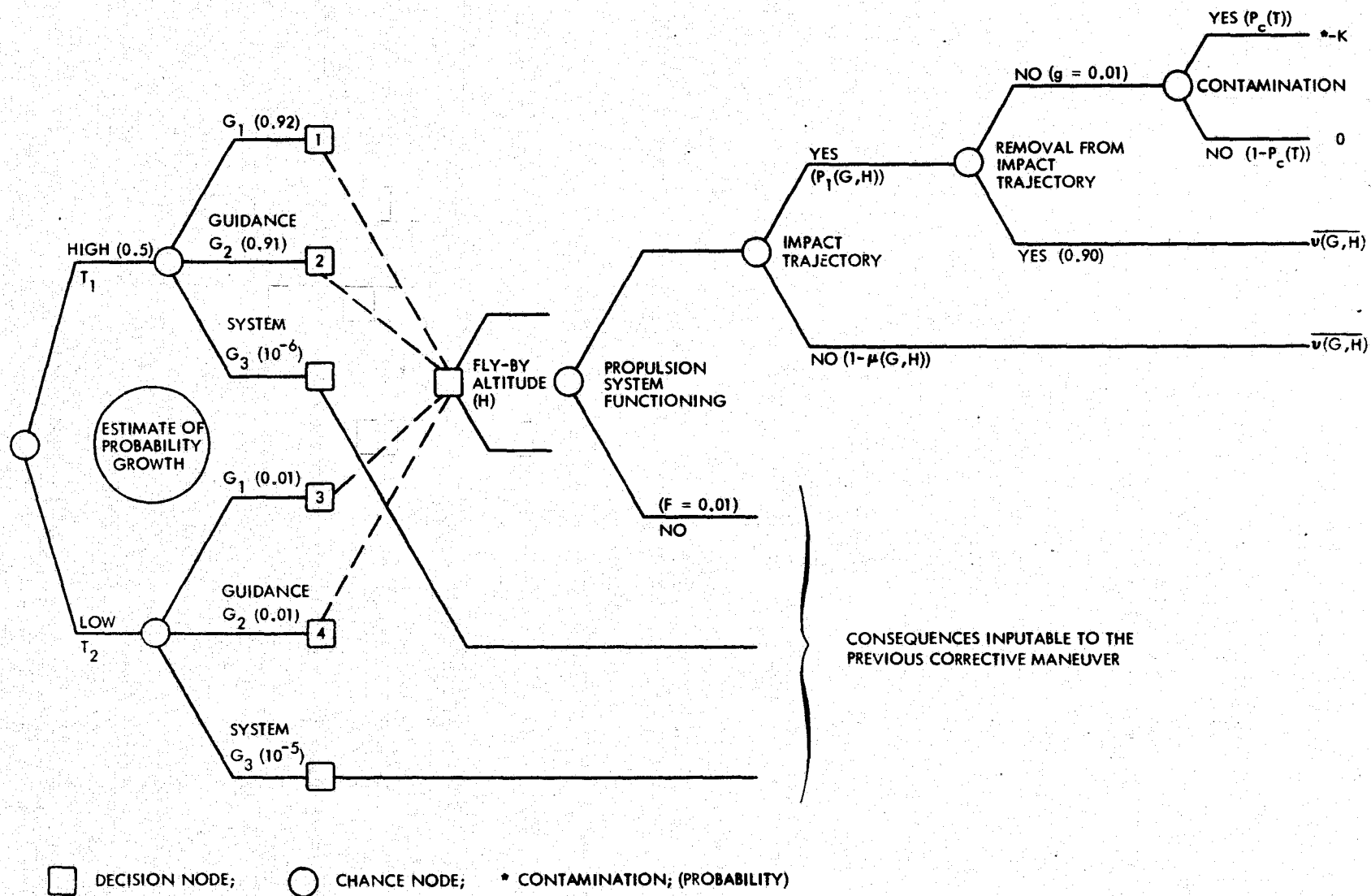
Fig. 1-2. Decision Tree for Final Pre-Uranus Maneuver

The probability of impact $(p_i(G, H))$ and the expected science value $(\overline{(V(G, H)})$ are shown as functions of the guidance system state and the fly-by altitude. In actual practice there would be more than fly-by altitude to consider but this is a simplified diagram.

The right-hand part of the diagram is entered by any of the four decision branches shown and the functions shown account for G and H. Two of the conditions shown result in no decision since there is no way to make the maneuver. These are the two conditions in which no guidance system is available (G_3). In these cases, the previous maneuver dictates the outcome.

The estimate of the probability of growth would be derived from spacecraft science measurements made prior to the last maneuver.

The approach outlined above would be useful for any mission even where inflight decisions based on new information are not a factor. The most difficult part of applying this technique is assessing the relative values of science measurements, spacecraft propellant, and other factors which are involved in the value of each outcome.

1.1.3 Future Activities

A planetary quarantine analysis of the probe phase of a Jupiter Orbiter Probe Mission will be performed. A final report for the atmospheric entry analysis will be prepared.

1.1.4 Presentations

Gonzalez, C., "Jupiter Entry Analysis," presented at the NASA Spacecraft Sterilization Technology Seminar, Cocoa Beach, Florida, August 1975.

1.1.5 Reference

Howard, R.A., North, D.W., and Pezier, J.P., "A New Methodology to Integrate Planetary Quarantine Requirements into Mission Planning with Application to a Jupiter Orbiter," Final Report, SRI Project MSD 3685, Stanford Research Institute, Menlo Park, Calif., August 1975.

SECTION II

NATURAL SPACE ENVIRONMENT STUDIES
(NASA No. 193-58-61-02)

Contents   Title and Related Personnel

Subtask A   EFFECT OF PLANETARY TRAPPED
para. 2.1   RADIATION BELT ON MICROORGANISMS

                             Cognizance: J. Barengoltz

                             Associate
                             Personnel: C. Meyers

Subtask B   EFFECT OF SOLAR WIND ON
para. 2.2   MICROORGANISMS

                             Cognizance: J. Barengoltz

                             Associate
                             Personnel: C. Apramian
                                          J. Brady (Bionetics)

Subtask C   EFFECT OF SPACE VACUUM ON
para. 2.3   MICROORGANISMS

                             Cognizance: M. Wardle
                                          E. Hagen

Subtask D   PROBABILITY OF GROWTH IN PLANETARY
para. 2.4   ATMOSPHERE AND SATELLITES

                             Cognizance: A. Ingersoll
                                          D. Taylor
                             Associate
                             Personnel: N. Divine
                                          C. Meyers

Subtask E   EFFECT OF SOLAR ELECTROMAGNETIC
para. 2.5   RADIATION ON MICROORGANISMS

                             Cognizance: M. Wardle

                             Associate
                             Personnel: D. Ross
                                          J. Brady (Bionetics)

2.1 EFFECT OF PLANETARY TRAPPED RADIATION BELT ON MICROORGANISMS

2.1.1 Subtask A Introduction

The objective of this subtask is to determine the effect of planetary trapped radiation belts on the survival of microorganisms associated with an unsterile spacecraft.

With fly-by missions now planned for Jupiter and Saturn and possible Jupiter orbiters and probes, the trapped radiation belts represents an environment lethal to microorganisms and would reduce a requirement for decontamination of spacecraft before launch.

The major components of planetary trapped radiation belts are electrons and protons. The approach of the present task is to evaluate possible biological effects of these belts by subjecting spacecraft microbial isolates to different energies, exposures, and dose rates of those particles.

2.1.2 Significant Accomplishments

The microbial radiation sensitivity modeling effort continued during this reporting period with the production of several computer programs comprising the major portion of the effort. The data being modeled is the high energy electron data previously obtained in this subtask (Refs. 1 and 2). Two of the programs preprocess the data prior to modeling (e.g. sort, compute averages, check for consistency, and reorganize the data). An extensive generalized program that fits any parametric model to the data was also developed.

Several dose-dependent models of the radiation sensitivity were investigated using the fitting program. These models include linear and quadratic models of log survival fraction and a two-parameter model that incorporates a term for repair processes.

Figures 2-A.1 and 2 show an example of the linear model for the response of <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u> to 2 MeV electrons at a flux of 10^{10} electrons/cm^{2}s. Circles represent the experimental data. The solid line represents the model and the dashed lines are the 95% confidence interval for
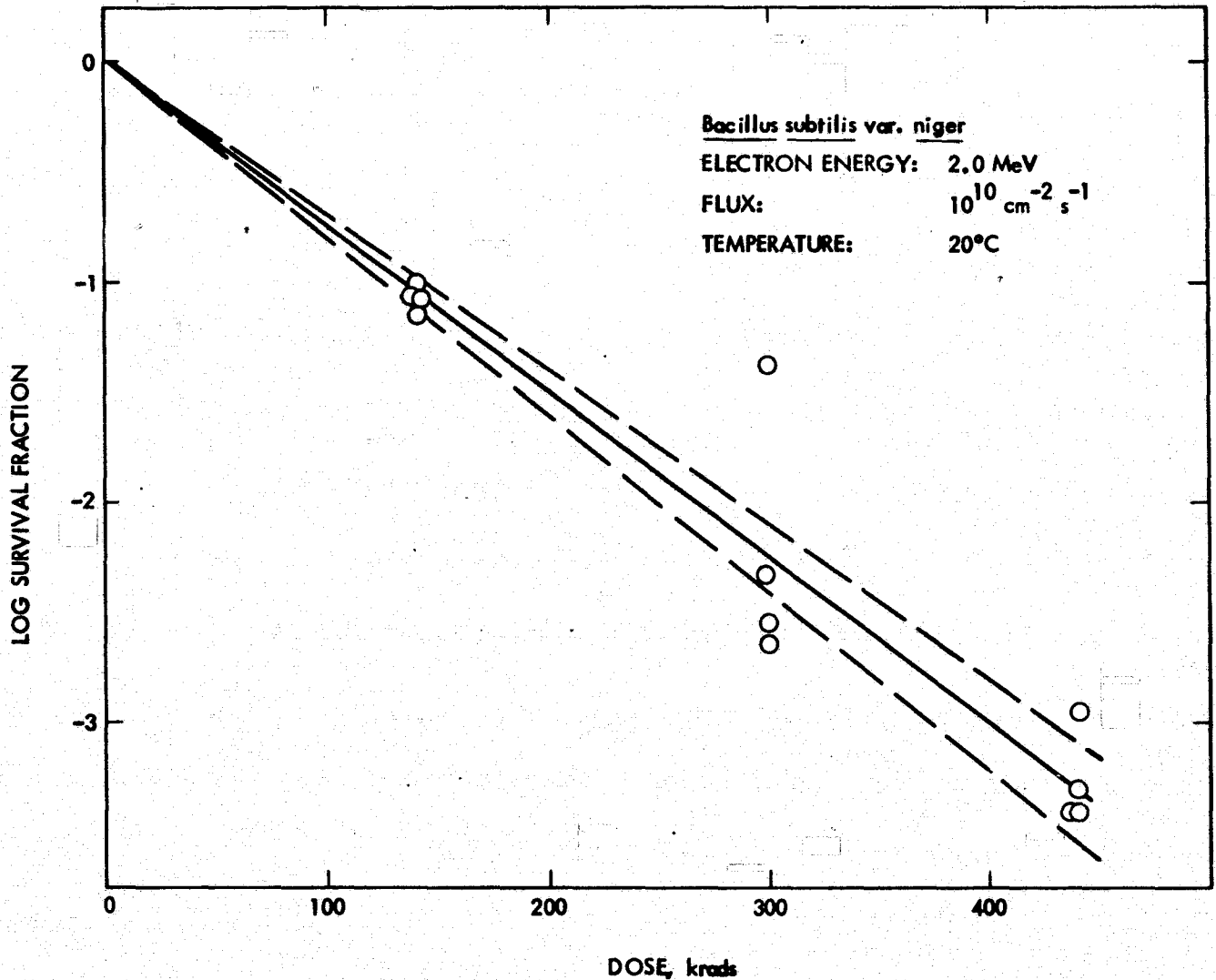
Fig. 2-A.1. Organism Survival Model at 20°C

the model. This linear model gives the least error over the data range of all the models thus far investigated. Survival dependence on other parameters has not yet been investigated but Figures 2-A.1 and 2 reflect a significant temperature dependence (Ref. 2).

2.1.3 Future Activities

The modeling effort will continue in the investigation of model dependence on other variables and the development of more extensive models.
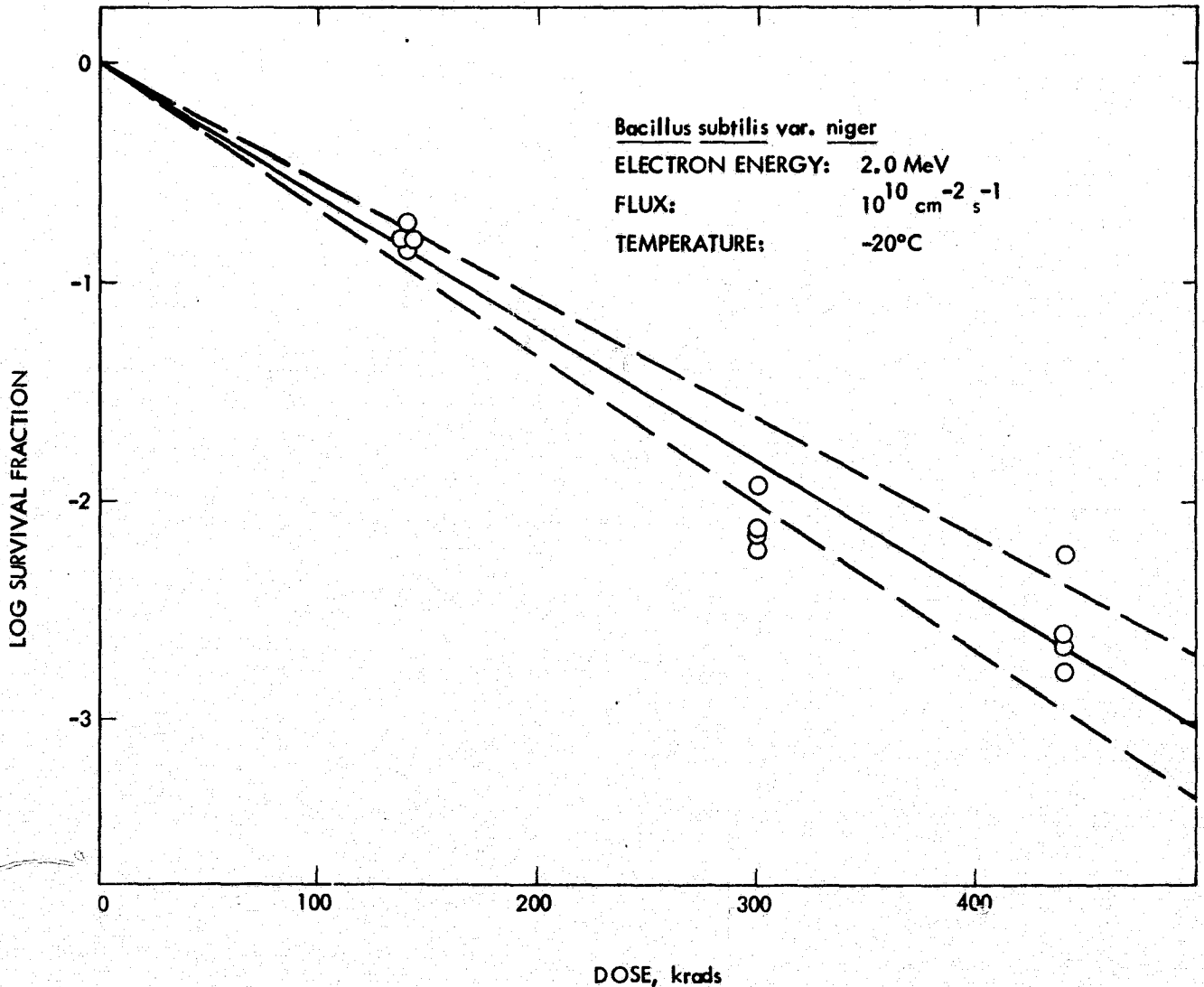
Fig. 2-A.2. Organism Survival Model at -20°C

A contract for a study to determine the effect of particle radiation on encapsulated microorganisms will be awarded. This study will provide data to be compared with data for microbes on a surface, obtained previously under this task. The effect of factors not modeled in convectional radiation transport calculations will be investigated.

The present radiation environment for Jupiter is provisional and will be recomputed as Pioneer 11 radiation measurements are incorporated into the radiation belt models. These updated models will then allow an evaluation of external radiation environments in terms of electron test data.

An analysis of the effect of secondary radiation will be performed with the use of the new models, the encapsulated microbe data, and the electron transport program (previously modified and adapted for use on the JPL Univac 1108 computer system).

Planning for the procurement of a study with high energy protons similar to those present in planetary trapped radiation belts will continue.

2.1.4   References

1.   Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January - 30 June 1973, JPL Doc. 900-636, pp. 2-1 to 2-9, 1973.

2.   Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July - 31 December 1973, JPL Doc. 900-655, pp. 2-1 to 2-12, 1974.

2.2   EFFECT OF SOLAR WIND RADIATION ON MICROORGANISMS

2.2.1   Subtask B Introduction

The objective of this subtask is to determine the effect of solar wind radiation on microorganisms associated with nonsterile spacecraft.

This study is directed towards determining the radiation in spacecraft-associated microbial burden attributable to solar wind radiation. The data obtained will be utilized to update probability constants in the assessment of mission planetary quarantine constraints.

2.2.2   Approach

In order to fulfill the objectives of this task, an initial test program has been established to investigate the effect of solar wind electrons on test microorganisms held in a vacuum. A literature survey indicates biological effectiveness for electrons with energy in excess of 1 keV. Measurements and models of the solar wind electron spectrum imply an upper limit to the energy range of interest at about keV. Parametric tests in this energy range, 1-5 keV, will be conducted at accelerated dose rates to permit typical mission doses (fluences) in acceptable test durations. Other radiation effects data indicate significant dose rate effects are unlikely. At each energy tests with varying doses will be performed to obtain survival curves.

The first formal experimental phase will consist of tests with MM'71 isolates (sporeformers and non-sporeformers) and Staphylococcus epidermidis (SE) and spores of Bacillus subtilis var. niger (BSN) as comparative organisms. A second phase will be an analogous program with naturally occurring microbial populations as samples.

In long range planning with the electron source, close-to-real time exposures and sequential exposures with varying energy to simulate the spectrum are being considered.

2.2.3   Significant Accomplishments

During this reporting period the formal test program has been completed for cultured organisms (Ref. 1). A series of tests with dilute specimens was also conducted to investigate organism self-shielding.

2.2.3.1 The Solar Wind Electron Source (SWES). The SWES system and its operation, including dosimetry, have been described in detail in a previous JPL Semi-Annual Review (Ref. 2) (Figure 2-B.1).

2.2.3.2 Test Conditions for Cultured Organisms. The irradiations of the pure cultured organisms have been conducted in vacuum ($<10^{-5}$ torr) at room temperature according to the test matrix Table 2-B.1. The electron fluences (doses) have been selected to include predicted fluences for a 1 year period at 1 AU heliocentric distance in an electron energy histogram, Table 2-B.2. Note that the test fluences decrease sharply with increasing energy to reflect the actual power law flux. The test fluxes (dose rates) represent a large acceleration over the models for the environment.

Each run (entry of the matrix) consisted of three specimens of each test organism. The test program included four replicates of each run.

2.2.3.3 Results and Discussion. The results of the formal test program are presented graphically in Figures 2-B.2 through 2-B.17. Certain features of the data may be noted:

1)   There is a large variation in the response for different organisms.

2)   Isolate No. 5, a micrococcus, is extremely resistant compared to isolate No. 4, a micrococcus, or Staphylococcus epidermidis. This factor causes rather large mean non-sporeformer (VGMEAN) survival fractions (Figures 2-B.5, 9, 13, and 17).

3)   An obvious plateau effect at high fluences occurs for survival fractions between 0.001 and 0.01 in the case of BSN and the mean sporeformers (SPMEAN) and about 0.1 in the case of mean non-sporeformers at all energies.

The plateau effect is tentatively explained by sample clumping and the resultant shielding of individual organisms. It is well known that electron in
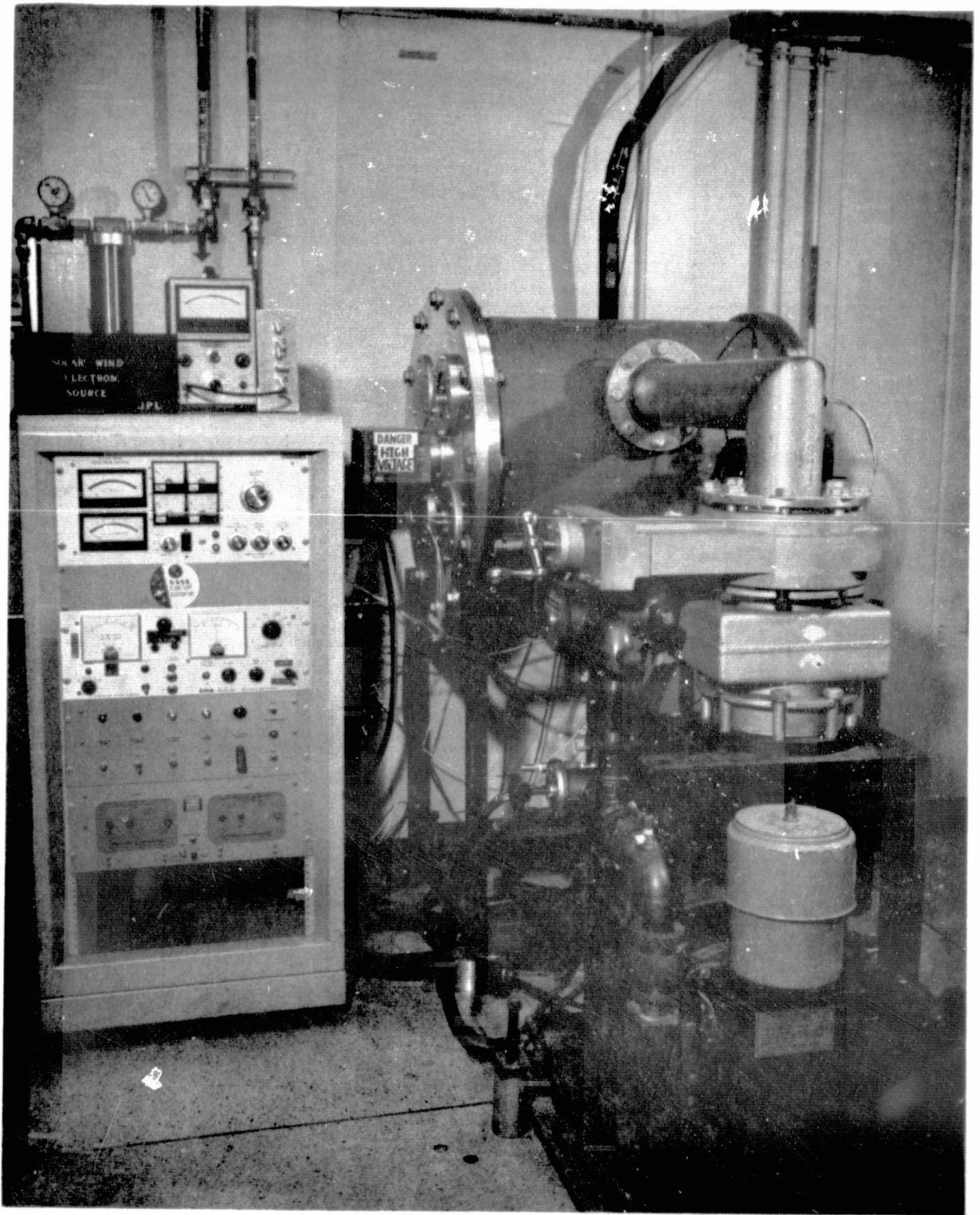
Fig. 2-B.1. Solar Wind Electron Source, Overview of System

Table 2-B.1. Electron Test Matrix for Cultured Organisms

Energy	Flux (e/cm^2s)					
1.5 keV		Fluence (e/cm^2)	5×10^{13}	1×10^{14}	5×10^{14}	1×10^{15}
	10^{12}	Duration	50s	100s	500s	1000s
2.0 keV		Fluence (e/cm^2)	2×10^{12}	1×10^{13}	5×10^{13}	2×10^{14}
	5×10^{10}	Duration	40s	200s	1000s	4000s
3.0 keV		Fluence (e/cm^2)	5×10^{11}	2×10^{12}	5×10^{12}	1×10^{13}
	10^{10}	Duration	50s	200s	500s	1000s
4.5 keV		Fluence (e/cm^2)	2×10^{11}	5×10^{11}	2×10^{12}	5×10^{12}
	5×10^{9}	Duration	40s	100s	400s	1000s

Table 2-B.2. Predicted Solar Wind Electron Fluence for 1 Year at 1 AU

Energy Interval keV	Model A Fluence[a] cm-^{2}	Model B Fluence[b] cm-^{2}
1.0 - 2.5	3×10^{12}	6×10^{12}
2.5 - 4.0	4×10^{11}	9×10^{11}
4.0 - 5.0	9×10^{10}	2×10^{11}

[a]based on:
Divine, T.N., "Interplanetary Charged Particle Environments," JPL TM 33-637 (1973).

[b]based on:
Montgomery, M.D., Bame, S.J., and Hundhausen, A.J., "Solar Wind Electrons: Vela 4 Measurements," Journal of Geophysical Research, Space Physics 73, No. 15, pp. 4999-5003 (1968).
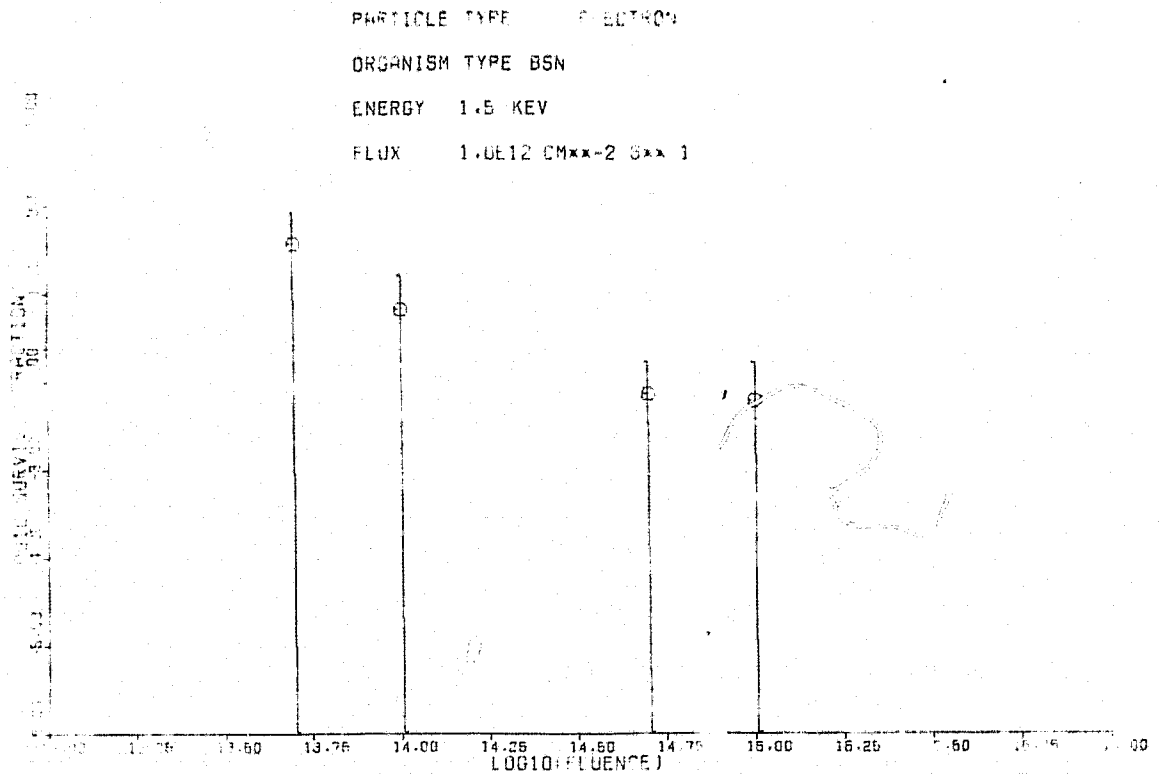
Fig. 2-B.2. Survival Fraction, Bacillus subtilis var. niger, 1.5 keV electrons at a flux of 1 x 10^{12} cm^{-2} 5^{-1}

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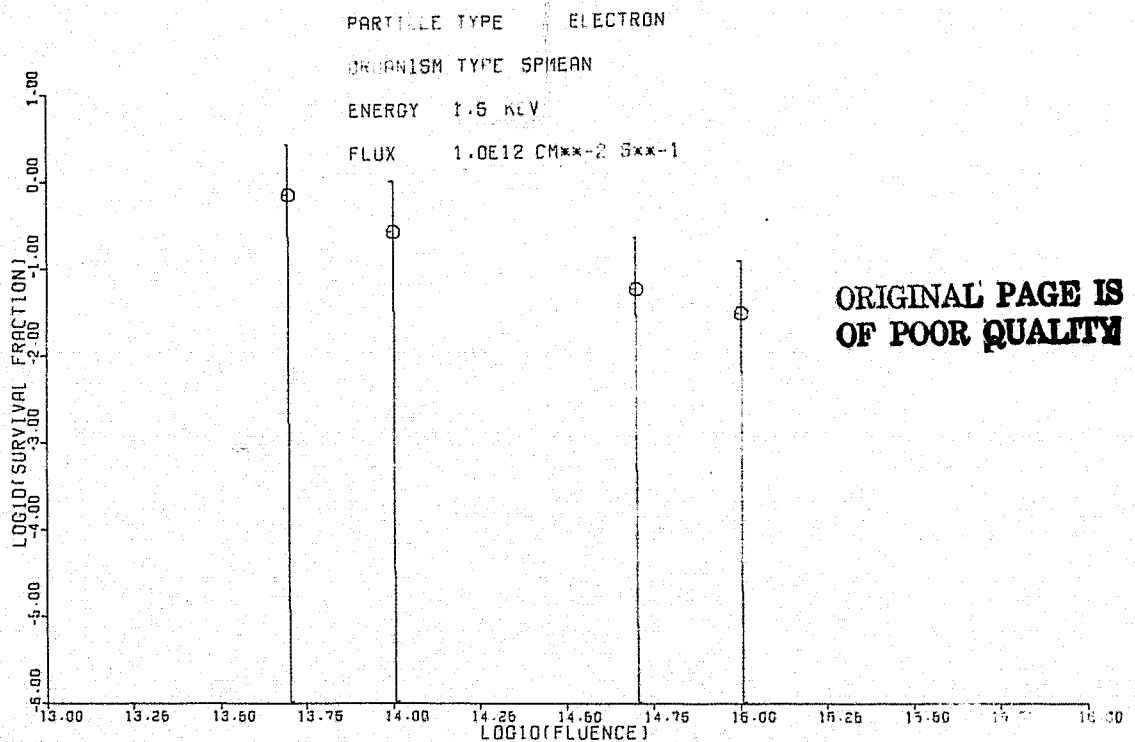
Fig. 2-B.3. Mean Survival Fraction, Sporeformers, 1.5 keV electrons at a flux of 1 x 10^{12} cm^{-2} 5^{-1}
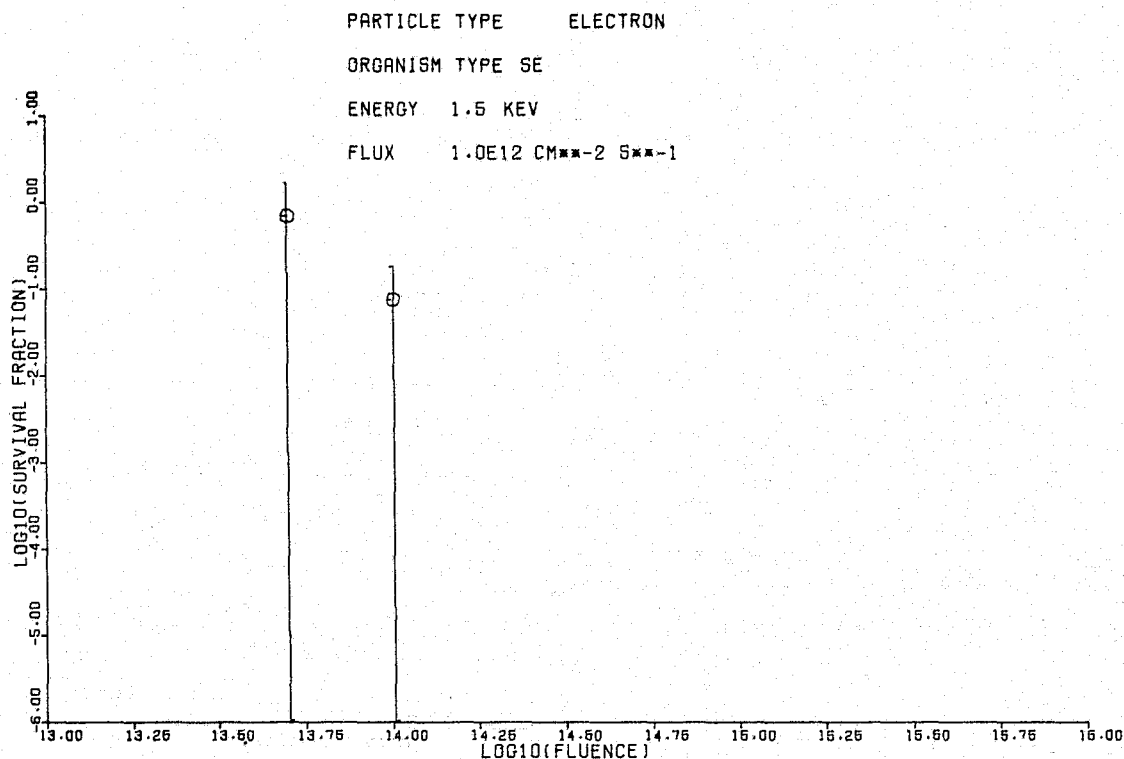
PARTICLE TYPE   ELECTRON

ORGANISM TYPE SE

ENERGY   1.5 KEV

FLUX   1.0E12 CM**-2 S**-1

Fig. 2-B.4. Survival Fraction, Staphylococcus epidermidis, 1.5 keV electrons at a flux of 1×10^{12} cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE VGMEAN

ENERGY   1.5 KEV

FLUX   1.0E12 CM**-2 S**-1
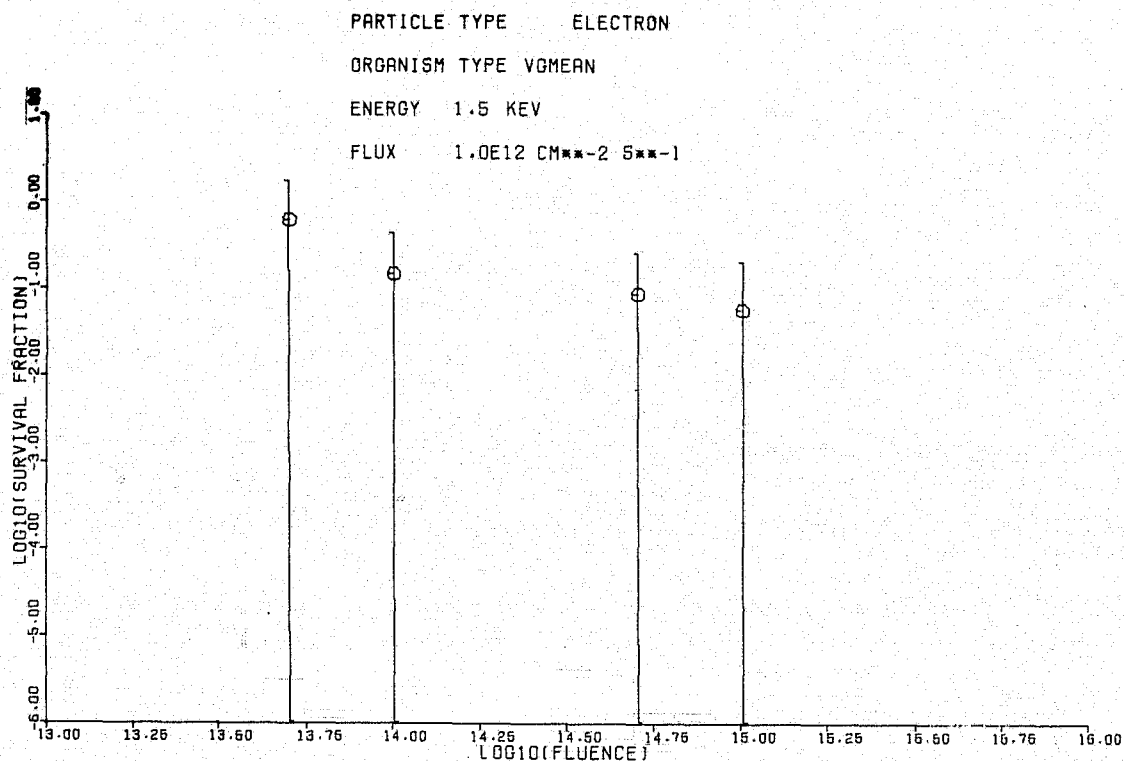
Fig. 2-B.5. Mean Survival Fraction, Non-sporeformers, 1.5 keV electrons at a flux of 1×10^{12} cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE BSN

ENERGY   2.0 KEV

FLUX   5.0E10 CM**-2 S**-1
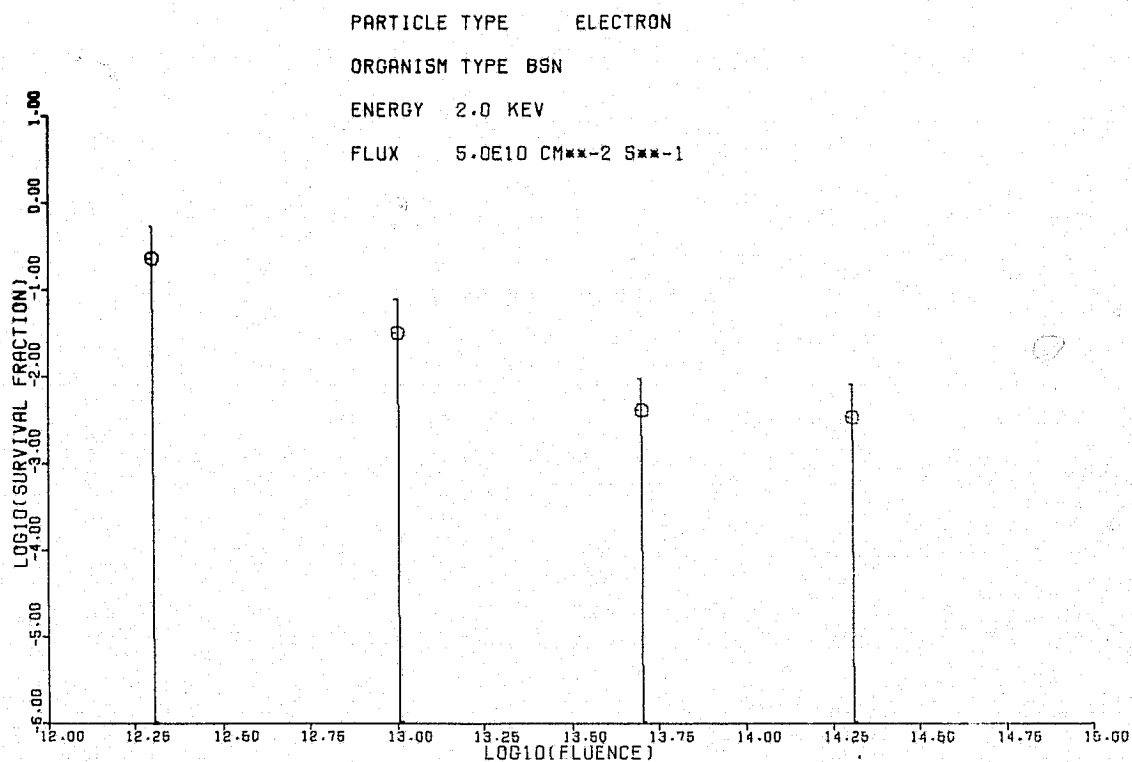
Fig. 2-B.6.   Survival Fraction, <u>Bacillus</u> <u>subtilis</u> vàr. <u>niger</u>, 2.0 keV electrons at a flux of 5×10^{10} cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE SPMEAN

ENERGY   2.0 KEV

FLUX   5.0E10 CM**-2 S**-1
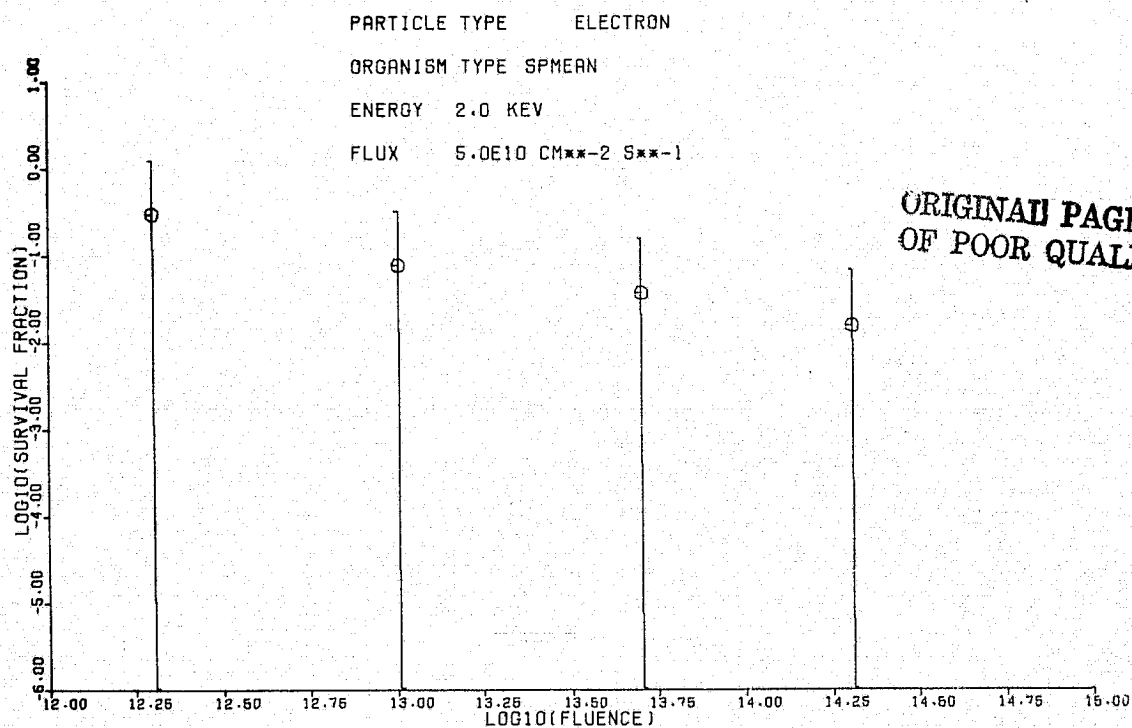
Fig. 2-B.7.   Mean Survival Fraction, Sporeformers, 2.0 keV electrons at a flux of 5×10^{10} cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE SE

ENERGY   2.0 KEV

FLUX   5.0E10 CM**-2 S**-1
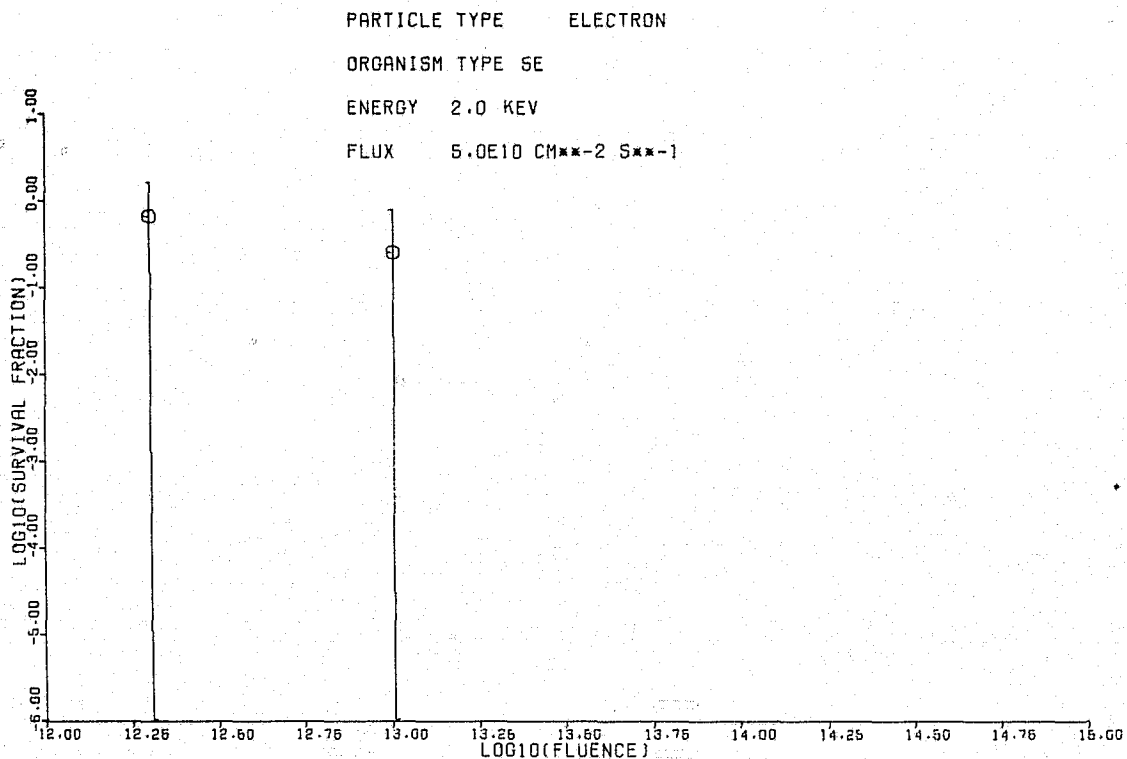
Fig. 2-B.8. Survival Fraction, Staphylococcus epidermidis, 2.0 keV electrons at a flux of 5×10^{10} cm^{-2} 5-1

PARTICLE TYPE   ELECTRON

ORGANISM TYPE VGMEAN

ENERGY   2.0 KEV

FLUX   5.0E10 CM**-2 S**-1
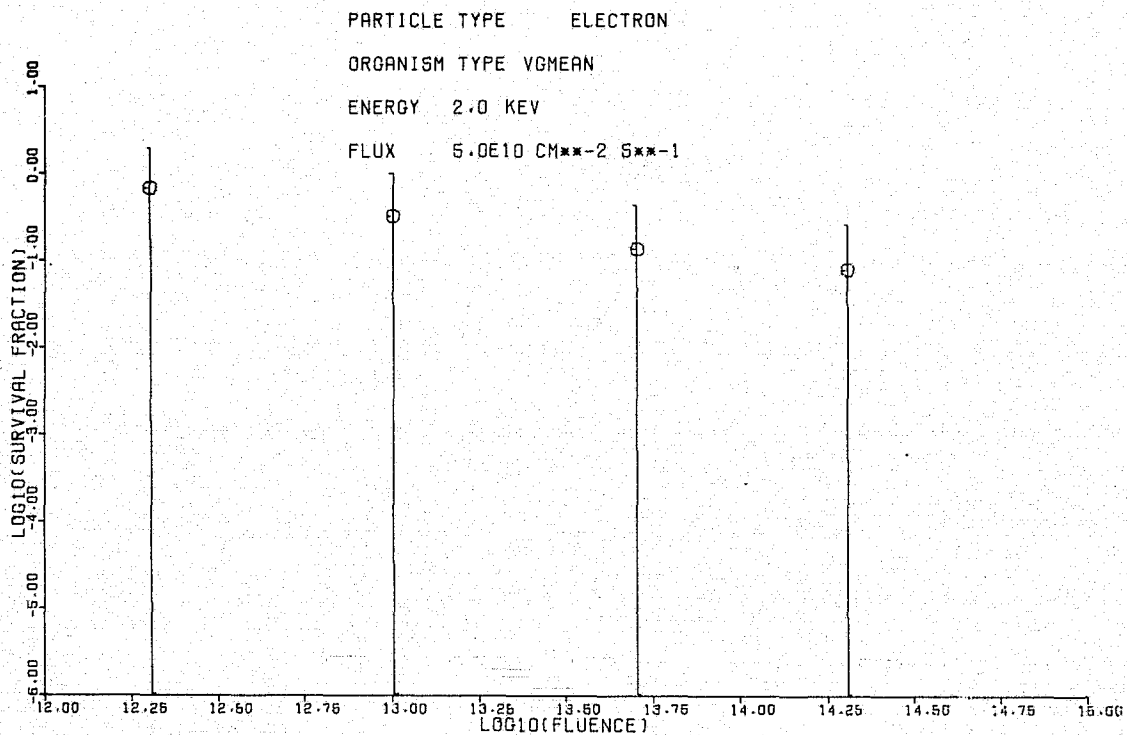
Fig. 2-B.9. Mean Survival Fraction, Non-sporeformers, 2.0 keV electrons at a flux of 5×10^{10} cm^{-2} 5-1

PARTICLE TYPE   ELECTRON

ORGANISM TYPE BSN

ENERGY   3.0 KEV

FLUX   1.0E10 CM**-2 S**-1
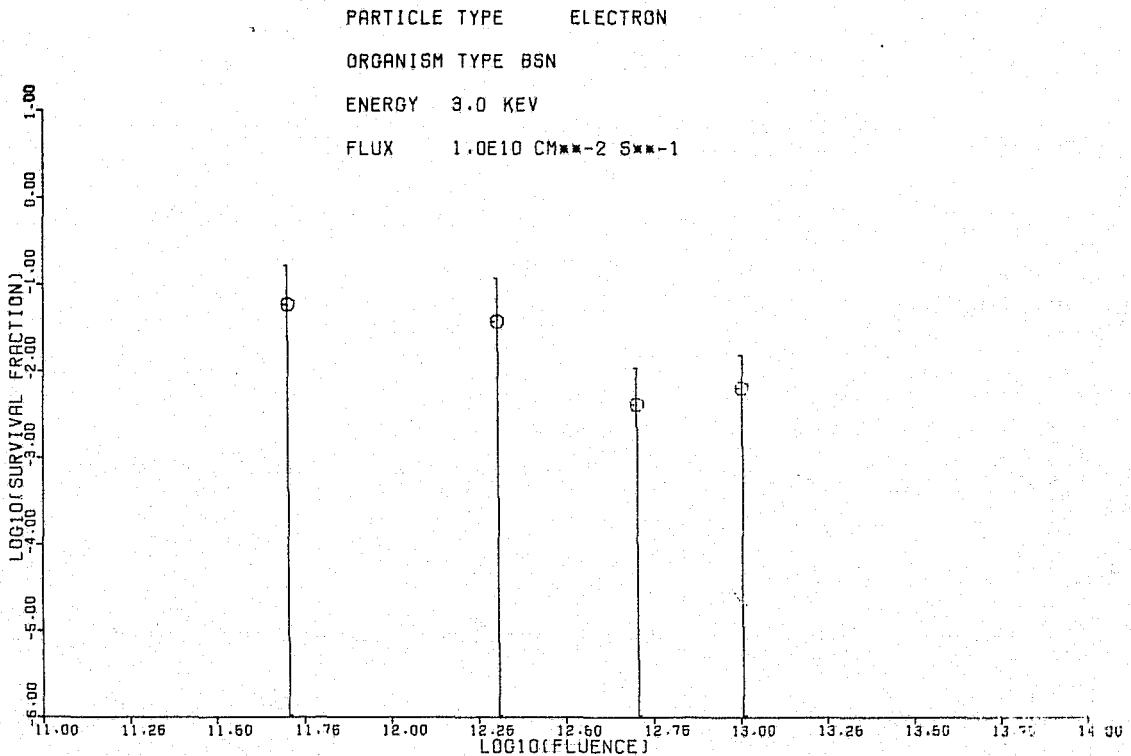
Fig. 2-B.10. Survival Fraction, <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u>, 3.0 keV electrons at a flux of 1 x 10^{10} cm^{-2} 5-1

PARTICLE TYPE   ELECTRON

ORGANISM TYPE SPMEAN

ENERGY   3.0 KEV

FLUX   1.0E10 CM**-2 S**-1

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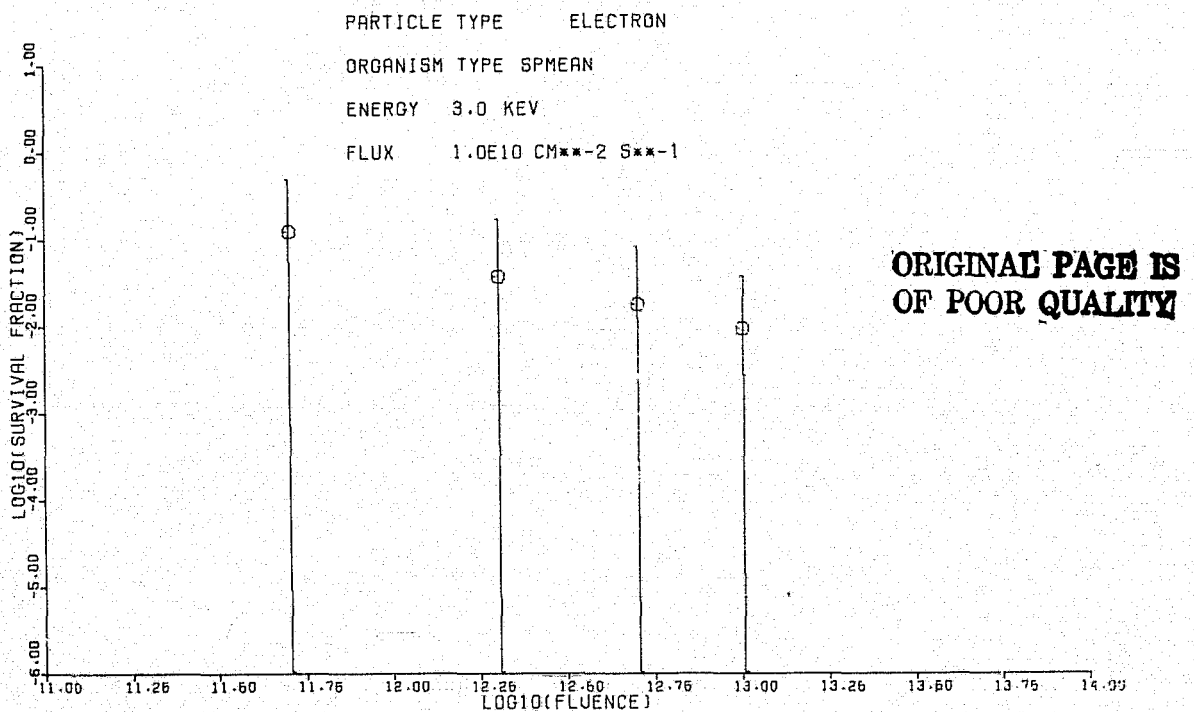
Fig. 2-B.11. Mean Survival Fraction, Sporeformers, 3.0 keV electrons at a flux of 1 x 10^{10} cm^{-2} 5-1
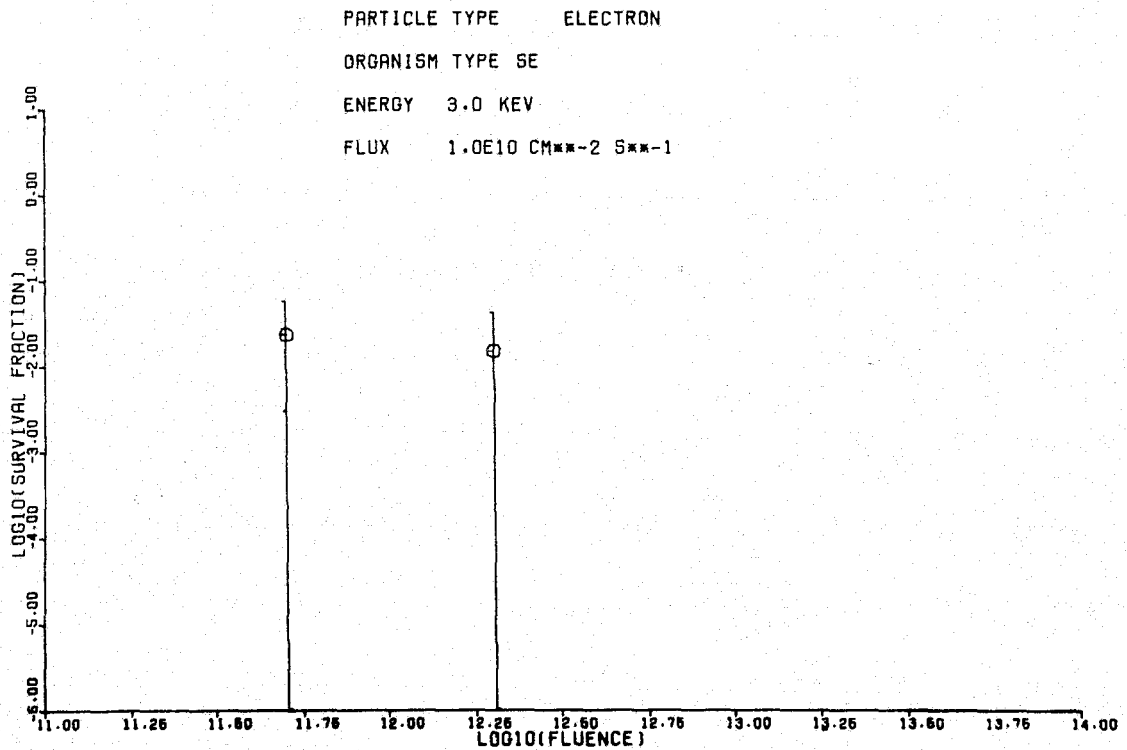
Fig. 2-B.12. Survival Fraction, <u>Staphylococcus</u> epidermidis, 1.5 keV electrons at a flux of 1 x 10^{10} cm^{-2} 5^{-1}
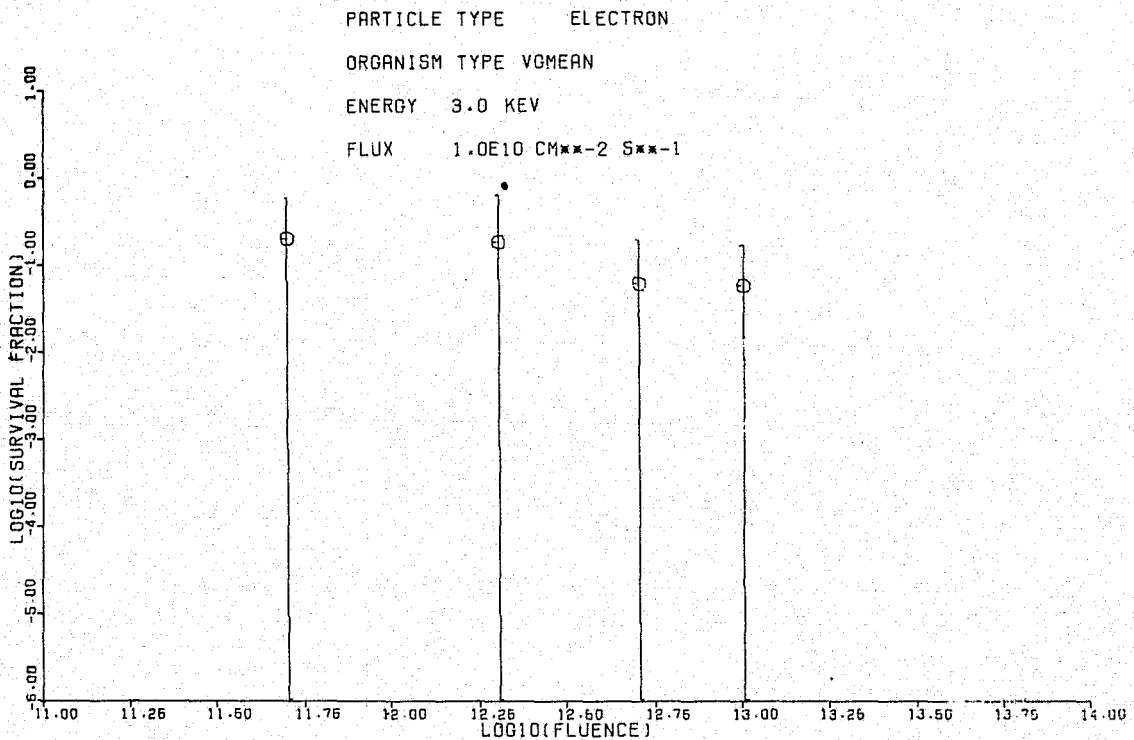
Fig. 2-B.13. Mean Survival Fraction, Non-sporeformers, 3.0 keV electrons at a flux of 1 x 10^{10} cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE BSN

ENERGY   4.5 KEV

FLUX   5.0E9 CM**-2 S**-1
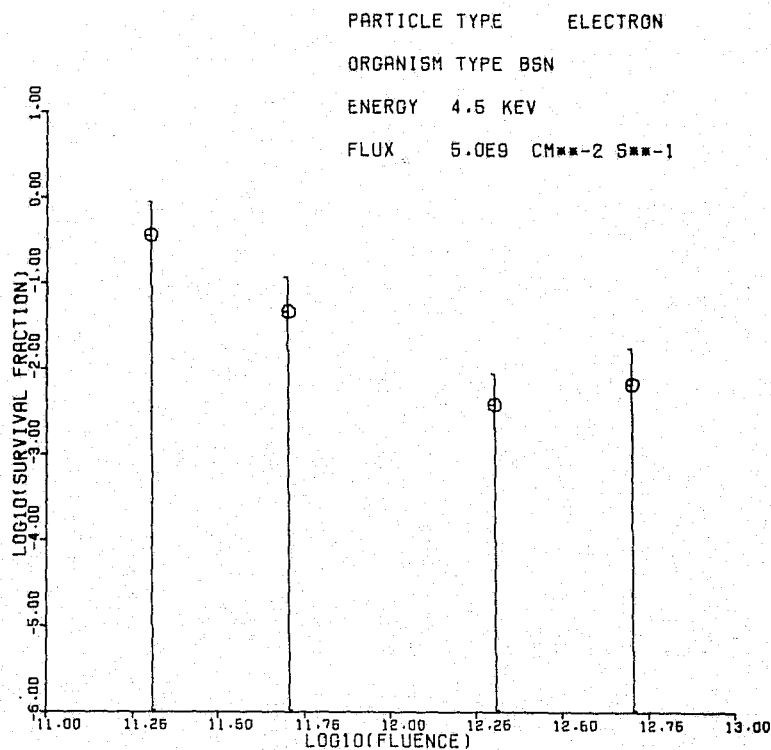
Fig. 2-B.14.   Survival Fraction, <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u>, 4.5 keV electrons at a flux of 5×10^9 cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE SPMEAN

ENERGY   4.5 KEV

FLUX   5.0E9 CM**-2 S**-1
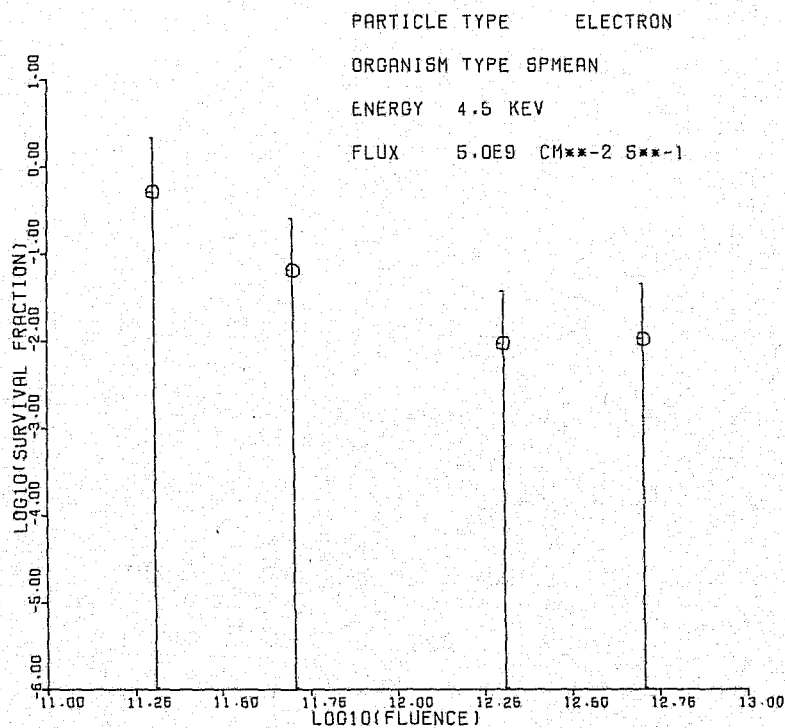
Fig. 2-B.15.   Mean Survival Fraction, Sporeformers, 4.5 keV electrons at a flux of 5×10^9 cm^{-2} 5^{-1}

PARTICLE TYPE   ELECTRON

ORGANISM TYPE

ENERGY   4.5 KEV

FLUX   5.0E9 CMxx-2 Sxx-1
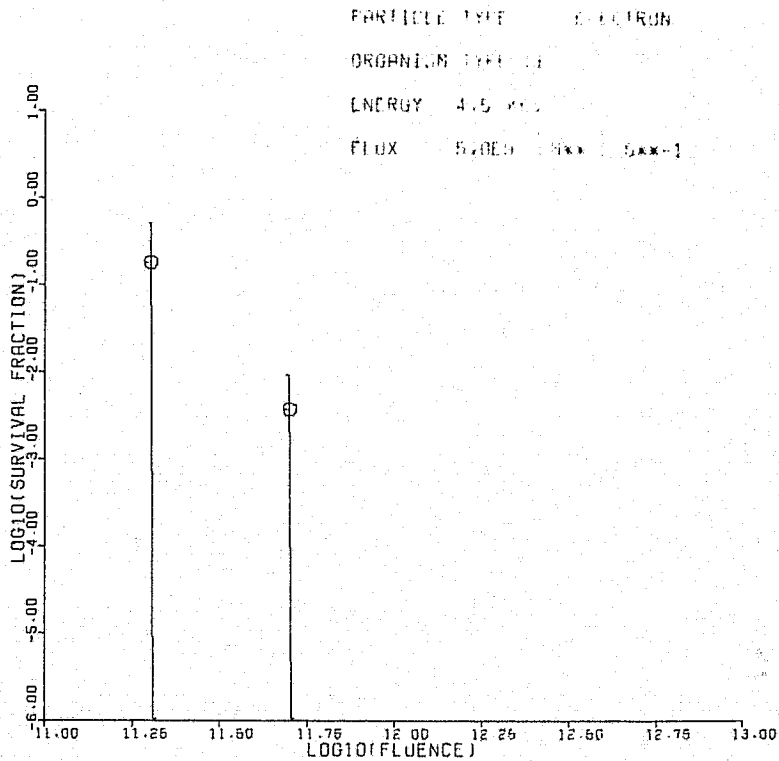
Fig. 2-B.16.   Survival Fraction, Staphylococcus epidermidis, 4.5 keV electrons at a flux of $5 \times 10^{10} cm^{-2} 5^{-1}$

PARTICLE TYPE   ELECTRON

ORGANISM TYPE VGMEAN

ENERGY   4.5 KEV

FLUX   5.0E9 CMxx-2 Sxx-1
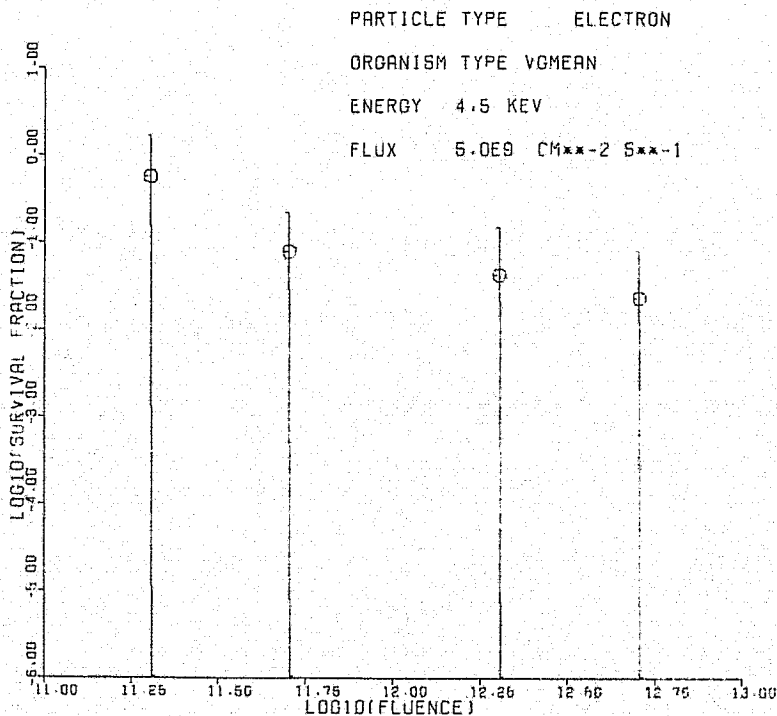
Fig. 2-B.17.   Mean Survival Fraction, Non-sporeformers, 4.5 keV electrons at a flux of $5 \times 10^9 cm^{-2} 5^{-1}$

the energy range studied cannot penetrate through an organism, e.g., the range of a 5 keV electron in organic material is only 0.57 μm (Refs. 3 and 4). Under this hypothesis, the minimum survival fraction is then interpreted as the fraction of cells which are shielded by at least one other cell. Since the test samples represent an extremely large density ($\sim 10^6$ cm^{-2}) compared to a real surface, this effect would render the present results very conservative.

Isolate No. 5, a micrococcus in a tetrad form, may be an exception in that any cell of the tetrad will typically be shielded by one or more of the others. This self-shielding may explain both the resistance of No. 5 to the radiation and the high plateau survival fraction for the mean non-sporeformers, dominated by the results for No. 5.

The results may also be summarized in terms of the D_{10} values, or the fluence required to produce a one order of magnitude reduction the survival fraction. Calculated values from the linear regression analysis for four fluence values are given in Figure 2-B.18. Because of the plateauing effect the D_{10} values represent over-estimates of the fluence required for the first order of magnitude reduction. Note That D_{10} for Bacillus subtilis (BS) as measured by Davis (Ref. 5) is considerably smaller than the present results, especially at the higher energies. Davis' values are for the first order of magnitude reduction since she innoculated only 50 microbes on a 1 cm^2 substrate to avoid clumping. A revision of our D_{10} values, to a fit for survival fractions greater than 0.02 only, yields excellent agreement with the published work.

In order to investigate the effect of sample self-shielding or clumping at the standard innoculations levels of 10^6 organisms on a 1 cm^2 substrate, additional tests at the 2 highest fluences were conducted with innoculations of 10^4. It was expected that the clumping would be significantly reduced while a minimum detectible survival fraction of 10^{-4} would be obtained. Thus the survival fractions at the highest fluences should be reduced from 10^{-2} toward 10^{-4}; i.e., the plateau effect should disappear, except for isolate No. 5. However, the results were negative. No statistically significant change was found.

At this point one must conclude either that clumping is not the cause of the plateau effect or that an innoculation of 10^4 is still clumped. Higher energy tests would investigate this matter in more detail.
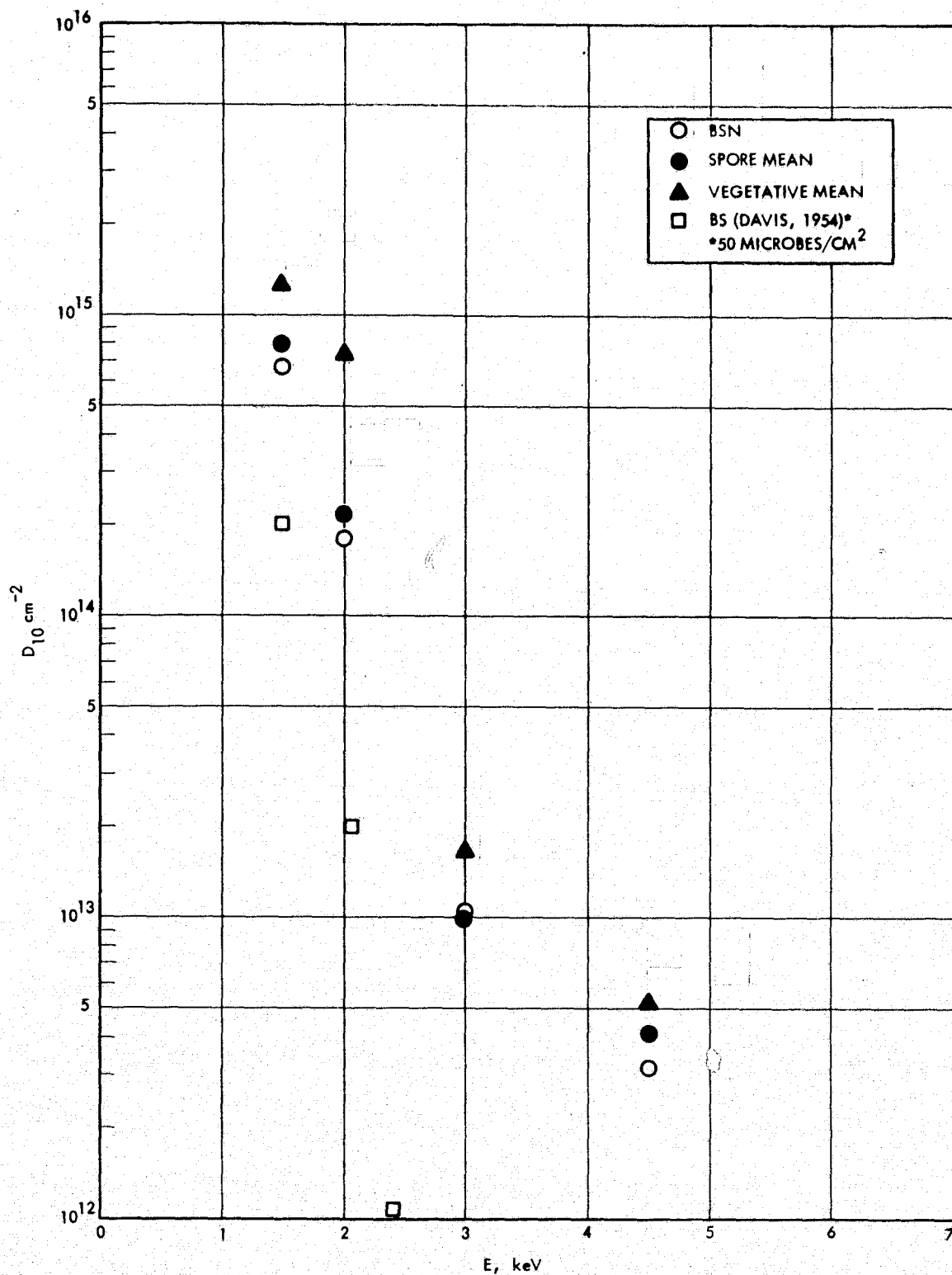
Fig. 2-B.18. D_{10} Values (Fluence) as a Function of Electron Energy

2.2.4 Future Activities

Formal tests with naturally occurring organisms collected on fall-out plates will be conducted.

The data from both the cultured and naturally occurring microbe tests will be modeled.

A study of the shielding factor discussed above will be undertaken. Plans call for more dilute innoculations and exposures at higher (more penetrating) electron energies.

2.2.5 Presentations

1. Barengoltz, J., "Effect of Solar Wind Radiation on Micro-organisms," presented at NASA Spacecraft Technology Seminar, Cocoa Beach, Fla., August 1975.

2. Barengoltz, J., "Effect of Solar Wind Radiation on Micro-organisms," a Center for Radiation Research Seminar, National Bureau of Standards, Washington, D.C., November 1975.

2.2.6 References

1. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January - 30 June 1975, JPL Doc. 900-715, 1975.

2. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July - 31 December 1974, JPL Doc. No. 900-701, pp. 2-4 through 2-10, 1975.

3. Lea, D.E., The Action of Radiation on Living Cells. Cambridge University Press, 1947.

4. Davis, M., Physical Review 94, pp 243, 1954.

5. Davis, M., Arch. Biochem. and Biophys. 48, pp 469-481, 1954.

2.3   EFFECT OF SPACE VACUUM ON MICROORGANISMS

2.3.1   Subtask C Introduction

The objective of this task is to determine the effect of extended exposure to space vacuum and spacecraft temperatures on the viability of microbial species representative of those found on spacecraft.

Earlier work in this task area concentrated on the study of bacterial isolates from MM'71 spacecraft (JPL Documents 900-597, 1972; 900-608, 1973; and 900-675, 1974). These isolates, along with Bacillus subtilis var. niger and Staphylococcus epidermidis (ATCC 17917), were exposed to a vacuum of 10^{-7} torr at temperatures of -40 to 75°C for durations from 7 to 187 days. In general, it was found that a significant vacuum/temperature effect occurred at temperatures of 40°C and greater for all durations studied. In addition, spores evidenced greater survival than did nonsporeformers.

Previous studies of the environmental microbiology of spacecraft assembly areas (e.g., Taylor, et. al. 1970, Dev. Indust. Micro. 11: 225-240), have indicated that approximately 90% of the naturally occurring microbes in such areas are nonsporeformers. Although technical difficulties exist in the investigation of the response of naturally occurring microbes to environmental stress, the significance of these populations is great in terms of quarantine considerations. It was therefore decided that the study of the vacuum/temperature effect on naturally occurring microorganisms would make more complete the understanding of this parameter of the natural space environment.

2.3.2   Significant Accomplishments

2.3.2.1 Experimental Activities.   Naturally occurring microbial fallout was collected in a cable assembly shop at JPL. The collection fixture was composed of 7.5 × 31.3 cm aluminum plates 3 mm thick (that simulated a spacecraft surface) to which Kapton foil heaters were bonded (on the reverse of the collection surface). Plates were exposed to the fallout environment for one week.

Simultaneous testing of pure cultured Bacillus subtilis var. niger (BSN) spores was also conducted. BSN spores were nebulized onto the

aluminum plates at a density representative of that exhibited by the naturally occurring populations (10^3 to 10^4 colony forming units ft^{-2}).

Eight plates were mounted onto a support frame using Teflon studs (for thermal isolation) with the following distribution: Two with naturally occurring microorganisms, two with BSN and four sterile controls. (Plates of each category were assayed (see below) to determine the initial population levels). The plates were then placed into the vacuum chamber and exposed to a 10^{-6} torr vacuum at 75°C (±3°C) for 7 days. After vacuum/temperature exposure, the plates were assayed on a class 100 bench using the agar contact technique: 16 samples being taken per plate. Samples were incubated at 32°C for up to 72 hours.

2.3.2.2 Results. Survival fractions as shown in Table 2-C.1 were calculated by taking the ratio of the mean number of colony forming units after vacuum/ temperature exposure (N_t) to the number initially on the plates (N_o). As can be seen there was a significantly greater resistance to the vacuum/temperature environment exhibited by the naturally occurring populations as compared to pure cultured. These survival fractions were derived using room controls in the calculation of N_o. Such controls represent plates that were seeded with the test populations and held at room conditions (23°C, 40-50% Relative Humidity for 7 days while the test plates underwent vacuum/temperature exposure. Another set of controls was assayed at the commencement of vacuum/temperature exposure. These N_o estimates, while identical to the aforementioned ones for BSN spores, provided lower survival fraction estimates (on an average, approximately 0.40) when used in the calculation of naturally occurring population

Table 2-C.1. Survival Fractions of Organisms Exposed to Space Vacuum (10^{-6} torr, 75°C, 7 days)

Test	Pure Cultured	Naturally Occurring
1	0.034 (±0.014)*	0.453 (±0.012)
2	0.008 (±0.007)	0.504 (±0.050)
*Standard Deviation		

survival fractions. This was most likely due to the large number of naturally occurring nonsporeformers that were rendered nonviable over the 7-day hold at room conditions. Hence, it was felt that, for naturally occurring microorganisms, the calculation of survival fractions using room controls provided a conservative estimate of the survivability of these populations.

The previous studies of BSN spores yielded an average survival fraction of 0.0002 (10^{-7} torr, 7 days, 75°C) which is significantly less than that observed in the present study. The discrepancy between the two experiments is most likely explained as a function of vacuum chamber design. The initial tests were conducted in a 6 in. internal diameter stainless steel chamber whereas the pure culture/naturally occurring tests utilized a 30 in. internal diameter chamber with a black (Liquid Nitrogen) cold wall (-185°C). It is quite possible that vacuum chamber wall characteristics must be considered in the definition of experiments designed to heat stress microorganisms. The temperature measurements of the substrate on which the microorganisms are located may not be an adequate specification of the thermal conditions of the microbial cells. The reflectivity of the 6 in. chamber's stainless steel walls may have led to the transfer of much of the radiant energy back through the cells and, combined with a lesser emissivity of the cells (relative to the substrate), produced a condition whereby their temperature was actually greater than that sensed for the substrate. It is hypothesized that the present experiment, which utilized a black cold wall, offered the converse situation; i.e., infrared energy was not significantly reflected from the wall and therefore the temperature of the microbial cells more closely approximated that of the substrate than in the previous tests. For a more complete discussion of this phenomenon see para. 4.2, "Evaluation of Vacuum/Heat Sterilization." The black cold wall system, while representing a condition that better simulates the space environment, probably alleviates the "wall phenomenon" complication and therefore provides for a more conservative estimate of the effect of the vacuum/temperature parameter of the natural space environment on microorganisms.

2.3.3   Future Activities

Naturally occurring microorganisms surviving vacuum/temperature tests will be identified.

2.4 PROBABILITY OF GROWTH IN PLANETARY ATMOSPHERES AND SATELLITES

2.4.1 Subtask D Introduction

The objectives of this subtask are to relate environmental parameters affecting microbial growth to conditions present in the atmospheres of Jupiter and Saturn, and to identify and study satellites of Jupiter and Saturn having possible biological interest.

2.4.2 Significant Accomplishments

The final report for the study "Structure and Dynamics of Jupiter's Atmosphere" performed under contract with California Institute of Technology by Dr. Andrew P. Ingersoll (Division of Geological and Planetary Sciences) has been submitted. Sections 2.4.2.1 through 2.4.2.5 present the conclusions of the study.

2.4.2.1 <u>Jupiter's Atmospheric Motions.</u> This study attempts to characterize motions in Jupiter's atmosphere over a range of scales from 10 km to 10^4 km. There are three possible atmospheric flow regimes: 1) thermal convection on a scale comparable to the atmospheric scale height (20-50 km), 2) baroclinic instabilities (possibly on a scale of order 650 km), and 3) large-scale general descent driven by radiative cooling. For the low end of this range, mixing-length theory of stellar convection zones describes the small-scale motions. The rapid rotation of the planet leads to a reduction of scale of the convection in the east-west direction, but no change in the temperature gradient or heat flux. In the intermediate range of atmospheric motions, quasi-geostrophic scaling is used to describe baroclinic instabilities. Latent heat effects are assumed to determine the vertical and horizontal temperature differences. For large-scale motions, a new theory stressing the importance of latent heat effects explains the east-west orientation of Jupiter's cloud bands and other qualitative features. Each of these three atmospheric flow regimes is capable of controling the residence times. All three regimes occur in the earth's atmosphere and might also occur in Jupiter's atmosphere. With an understanding of the physical processes driving each regime, it is possible to scale the mixing rates and residence times to Jovian conditions.

2.4.2.2 <u>Thermal Convection</u>.   Mixing-length theory describes small-scale thermal convection. For the Earth, the derived vertical velocities are about 7 m/s, which is typical of a small thunderstorm. The corresponding residence time (defined as the time needed to travel one scale height, about 9 km) is approximately 1/2 hr. For Jupiter, the same theory gives a residence time of about 10 hr (scale height about 42 km). These estimates are derived from the formulae

$$ W \sim \left(\frac{FR}{\mu \rho c_P} \right)^{1/3} \quad , \quad H = \frac{RT}{\mu g} \quad , \quad \nu \sim W/H \qquad (1)$$

where W is vertical velocity, H the atmospheric scale height, F the vertical heat flux, R the universal gas constant, μ the molecular weight, ρ the density, T the temperature, c_P the specific heat of the gas, and g the acceleration of gravity. The values of parameters used in these formulas are given in Table 2-D.1. The residence time is approximately H/W. The difference between the theoretical residence times on the Earth and those on Jupiter is due to the lower heat flux on Jupiter and the greater specific heat of hydrogen gas compared to air.

The fact that the residence time is not short compared to the Jovian day means that Coriolis forces play an important part in the dynamics. Such a situation has not been studied in other atmospheres, so it is difficult to predict what effect Coriolis forces will have on the small-scale convection. One possibility is that the east-west scale of the motion and east-west velocity are reduced but with no change in the vertical velocity and vertical circulation time. The other possibility is that Coriolis forces inhibit the vertical circulation and lengthen the residence times. The 10 hr estimate given in the preceding paragraph is based on the first possibility.

For the Earth's atmosphere, mixing-length theory leads to a gross under-estimate of mean tropospheric residence times. This is because the Earth's atmosphere is stably stratified on the average, meaning that the lapse rate of temperature is less than the dry adiabatic lapse rate. Small-scale convection can occur when the air is saturated, in which case the critical lapse rate becomes the wet adiabatic lapse rate, which is less than the dry adiabatic lapse rate. Also, large-scale convection driven by horizontal temperature differences tends to occur even when the air is unsaturated and stably stratified.

Table 2-D.1. Adopted Values of Parameters

Symbol	Definition	Value
μ	mean molecular weight	2.22
R	universal gas constant	8.32×10^7 erg $(°K \text{ mole})^{-1}$
c_P	specific heat at constant pressure	1.25×10^8 erg $(°K \text{ gm})^{-1}$
P_2	reference pressure (cloud base)	4.5 bar
λ_r	reference latitude	30 degrees
T	temperature at cloud base	269°K
ρ	density at cloud base	4.4×10^{-4} gm cm^{-3}
g	gravitational acceleration	2400 cm sec^{-2}
Ω	planetary rotation rate	1.76×10^{-4} sec^{-1}
r	planetary radius	71,400 km
f	Coriolis parameter $2\Omega \sin \lambda_r$	1.76×10^{-4} sec^{-1}
β	$(1/r) \, df/d\lambda = 2\Omega (\cos \lambda_r)/r$	4.27×10^{-14} (cm sec)$^{-1}$
L	(width of belt-zone pair)/(2π)	2500 km
H	pressure scale height $RT/\mu g$	42 km
U	zonal velocity amplitude	30 m sec^{-1}
F	average internal heat flux	6.5×10^3 erg cm^{-2} sec^{-1}
α_w	molar mixing ratio of water	0.96×10^{-3}
E_w	specific latent heat of water	675 cal gm^{-1}
δT_L	temperature change $\alpha_w \mu_w E_w/(\mu c_P)$	1.76°K

2.4.2.3 <u>Baroclinic Instabilities.</u> The mean state of the Earth's atmosphere at mid-latitudes, in which there are large horizontal temperature gradients, is said to be baroclinic. The large-scale convective motions that are driven by these gradients are called baroclinic instabilities. These baroclinic instabilities convect heat poleward and upward, and seem to account for the mean

tropospheric residence times at mid-latitudes. Residence times are still of order H/W, but now W is given by

$$W \sim H U^2 / \left(L_D^2 \Omega \right) \quad , \tag{2}$$

where U is the typical difference in horizontal velocity between the bottom and top of the atmosphere, Ω is the Earth's angular velocity of rotation, and L_D is the radius of deformation, given by

$$L_D^2 \sim \frac{RH}{\mu \Omega^2} \left(\frac{dT}{dZ} + \frac{g}{c_P} \right) \quad . \tag{3}$$

For the Earth, L_D is about 10^3 km, and residence times computed from Eq. (2) are about 1 week. For Jupiter, it is difficult to estimate L_D because the factor in parentheses in Eq. (3) is unknown. This factor is the difference between the dry adiabatic lapse rate and the actual lapse rate, evaluated at levels where the horizontal temperature gradient is largest. A conservative estimate is obtained by assuming

$$1/2 H \left(\frac{dT}{dZ} + \frac{g}{c_P} \right) \approx \frac{\mu_w L_w \alpha_w}{\mu c_P} \approx \delta T_L \approx 1.8°K \quad , \tag{4}$$

where L_w is the specific latent heat of water vapor, μ_w the molecular weight of water, μ the molecular weight of the Jovian atmosphere, and α_w is the molar mixing ratio of water in the deep atmosphere. Eq. (4) is based on the assumption that the difference between the actual lapse rate is of the same order as the difference between the wet and dry adiabatic lapse rates. Only water contributes significantly to this difference, assuming Jupiter is close to solar composition.

Using this estimate for Jupiter, we obtain values of L_D around 650 km and residence times of 1 day or more. The estimate of residence times from baroclinic instability theory is highly uncertain, although the numbers are only slightly longer than those from mixing-length theory. Given our ignorance about the portions of Jupiter's atmosphere below the cloud tops, it is best to adopt the estimate 0.3 to 30 days for the time to travel one scale height. The

order-of-magnitude uncertainty is warranted because of our lack of knowledge of which flow regimes occur in Jupiter's atmosphere. Parameters of small- and intermediate-scale motions are summarized in Table 2-D.2.

Table 2-D.2. Parameters of Small- and Intermediate-Scale Motions

	Dry Convection	Moist Convection	Baroclinic Instability
Horizontal scale	10 - 100 km	10 - 100 km	10^2 - 10^3 km
Vertical scale	10 - 100 km	10 - 100 km	20 - 200 km
$(\Gamma_a - \Gamma)/\Gamma_a$	-10^{-5}	10^{-2}	10^{-2}
Horizontal velocity	0.5 - 5 m/s	0.5 - 5 m/s	5 - 50 m/s
Vertical velocity	0.5 - 5 m/s	0.5 - 5 m/s	1.5 - 150 cm/s
Residence time	0.1 - 1 day	0.1 - 1 day	0.3 - 30 day
Regions of occurrence	Entire globe $P \geq 5$ bar	Zones, red spots, $P \leq 5$ bar	Belt-zone edges, $0.5 \leq P \leq 10$ bar
Conditions for occurrence	Superadiabatic lapse rate	Saturated vapor	Horizontal temperature gradient
Analogue system	Stellar convection zones	Cumulus convection	Cyclones and anticyclones
Theoretical framework	Mixing-length theory	Mixing-length theory	Quasi-geostrophic theory

2.4.2.4 Radiative Cooling. A third direct estimate of residence times is based on the analogy between the belts of Jupiter and the trade-wind belts of the Earth. Both are clear regions in which large-scale sinking is regulated by the rate at which air cools by infrared radiation. The vertical velocity is computed from

$$\frac{dT}{dt}\bigg|_{rad} \sim W \left(\frac{dT}{dZ} + \frac{g}{c_P}\right) \quad , \tag{5}$$

where the left side is the rate of temperature change due to radiative cooling. For the Earth's tropics, residence times on the order of 1 month are common, and seem to be controlled by this process. For Jupiter, using Eq. (5) for the difference of lapse rates, we obtain residence times of at least one month. At deeper levels, where radiative cooling rates are smaller, the residence times are one year or longer.

The above estimate, obtained from Eq. (5), may be misleading from the point of view of planetary quarantine. If all the air which sinks in the belts is eventually mixed to great depths before it is recycled into the centers of rising motion, no suspended organisms will survive. This is a likely possibility, because the air which sinks in the belts will probably not move latitudinally until it reaches the levels where small-scale convection can provide rapid exchange of angular momentum. Thus air which is moving latitudinally from the belts to the zones is likely to be exchanged with air from deeper levels on a time scale that is controlled by small-scale convection. The fraction of air parcels that will survive this trip without being exchanged is of order

$$\exp\left[-\nu \, \Delta t\right]$$

where ν^{-1} is the residence time for small-scale convection and Δt is the circulation time from belts to zones. If ν^{-1} is less than 1 month and Δt is one year or more, the surviving fraction will be negligible. This estimate of Δt follows if the vertical scale for the return flow from belts to zones is as great as that for the descending flow in the belts. In both cases, the vertical scale is likely to be about one scale height.

The extreme dryness of the belts is another factor that makes them biologically uninteresting. Spectroscopic measurements indicate that the relative humidity at the 300°K level is about 10^{-3}. As the air sinks to lower levels, the relative humidity will decrease further. In this sense, the belts are anaomalous. The parts of the planet where both water and favorable temperatures exist are also characterized by active convection and short residence times. Thus, the effective residence time for the entire planet is one month or less.

The slowness of the large-scale circulation does not necessarily imply long residence times for individual fluid elements. Except in the descending portion of the cycle, where the flow is relatively uniform, the large-scale circulation is just the average of a much more vigorous small-scale motion. Small-scale mixing does affect the large-scale motions, however, by introducing a drag force between levels, as well as a lateral diffusion coefficient. Therefore, an attempt has been made to model the large-scale motions in order to find the best values of these mixing parameters.

The theory is for two moving layers above a stationary deep atmosphere. Horizontal temperature and pressure gradients exist in the upper layer (pressures 0 to 5 bar) as a result of latent heat and radiative heat exchange. The associated horizontal flow in the upper layer induces frictional stresses and flow in the lower layer (pressures 5 to 10 bar). Horizontal convergence in the lower layer leads to an increase in the water vapor concentration and an associated increase in the heating rate of the upper layer. The theory successfully accounts for the east-west orientation of belts and zones and the observed relation between temperature, horizontal flow and cloud height.

There are two time constants which enter in the theory. The first is the time for momentum exchange between the two layers and for lateral diffusion over a distance L_D. We associate this mixing with small- and intermediate-scale processes (Table 2-D.2), and denote the rate constant by ν. The second is the time for the vertical temperature gradient to adjust to changes in the water vapor mixing ratio below the clouds. This time constant, which we denote by τ, is likely to be one year or more. Its value is assumed to be greater than ν^{-1}. An important result of the theory is that the spacing of the belts and zones is approximately equal to the length L, where

$$L = L_D (\nu \tau)^{1/2} \tag{6}$$

with L_D given by Eq. (3). In principal, this result could be used to provide an indirect estimate of τ and ν, since the belt-zone spacing (about 10^4 km) is directly observable. For $\tau \approx 2$ years, $L_D = 650$ km, and $L = 2500$ km (Table 2-D.1), equation (5.6) gives $\nu^{-1} \approx 2$ months. However, there are many uncertainties in the theoretical model, and the value of τ is highly uncertain. Nevertheless, this model suggests that residence times may be longer than 1 month.

2.4.2.5 Summary. The time for a fluid particle to travel one scale height upward or downward in Jupiter's atmosphere may be anything from a few hours to a few months. Vertical transport properties, e.g., the time taken for a particle to move one scale height, appear to be controlled by small- and intermediate-scale motions. Small-scale convection gives mixing times of order 10 hours. Baroclinic instability gives times in the range 1 to 30 days. The theory of the large-scale motions gives an estimate of the mixing time due to small- and intermediate-scale motions of order 1 month or longer. Analogy with the Earth's atmosphere suggests that small-scale convection may not be the dominant mixing process and, therefore, that the mixing time for Jupiter may be one month or more. The uncertainty in the estimate of residence times is due partly to our ignorance of which flow regimes are dominant below the observable cloud tops and partly to our ignorance of the processes themselves.

2.4.3 Future Activities

A study analyzing the atmospheric dynamics of Saturn and Uranus in order to estimate the probability of growth on these planets is being considered.

2.4.4 Presentations

Ingersoll, A., "Dynamics of Jupiter's Atmosphere," presented at 7th Annual Meeting, American Astronomical Society Division for Planetary Sciences, Austin, Texas, 30 March - 3 April 1976.

2.4.5 Publications

Ingersoll, A. P., "The Atmosphere of Jupiter," to be published in Space Science Reviews, 1976.

Ingersoll, A. P., "Jupiter: Theory of Large-Scale Flow Driven by Latent Heat Exchange," submitted March 1976 to Journal of Atmospheric Sciences.

2.5   EFFECT OF SOLAR ELECTROMAGNETIC RADIATION ON MICROORGANISMS

2.5.1   Subtask E Introduction

The objective of this task is to estimate the effect of solar electromagnetic radiation (SER) on the survival of microbial populations in a space environment.   Efforts will be addressed to the investigation of the photobiological effect of SER in a fashion that permits direct transference of the results to considerations of planetary quarantine.   Such information will enable the updating of probability constants in the assessment of applicable planetary quarantine constraints for a mission.

2.5.2   Approach

The approach for this task involves the subjection of test species to SER in a manner that will yield interpretive data on the response of spacecraft biocontaminants to the SER of space.   Primarily, this entails the high vacuum irradiation of microorganisms, pure cultured and naturally occurring, with broad spectrum SER (far ultraviolet through infrared).   Pure cultured species are studied to define the effect of SER under different dose, dose rate and temperature conditions.   Naturally occurring populations are collected and exposed to SER in a natural state; i.e., no laboratory treatment of the organisms is instituted prior to test environment exposure.

2.5.3   Significant Accomplishments

Previous studies of naturally occurring organisms collected as fallout on Viking type solar cells indicated a greater resistance of these organisms to SER as compared to pure cultured spore and nonsporeformer MM'71 isolates. Therefore, to better understand the nature of this enhanced resistance, naturally occurring isolates were prepared in pure culture for comparative testing with MM'71 isolates.   Four nonsporeformers (Micrococcus spp.) and four sporeformers (Bacillus spp.) were selected for study.

Isolated colonies of nonsporeformers were obtained on quadrant streak plates.   These colonies were then inoculated into 10 ml of sterile Trypticase Soy Broth (BBL) and incubated at 37°C for 24 hours.   0.2 ml of the broth

suspension was then transferred to Trypticase Soy Agar (TSA) slants. Slants were incubated at 37°C for 48 hours and stored at 4°C. Two days prior to radiation exposure, 10 ml of sterile TSB was added to a slant and the cells were washed from the agar surface with 2 ml amounts of the suspension being added to each of two petri plates containing hardened TSA. TSA plates were incubated for 48 hours at 37°C. On the day of the test, the 48-hour lawns were harvested as follows: (1) 5 ml of sterile distilled water was added to the TSA plates (two for each organism); (2) The suspended organisms were transferred to 50 ml centrifuge tubes; (3) The cells were centrifuged at 7710 RCF for 10 minutes followed by 4 additional 20 ml washings of the pellet. The final suspension was then added to 4 ml of sterile distilled water until, as determined by absorbence readings at 500 nm, approximately 2×10^8 organisms per ml were obtained. Subsequent steps in the experimental protocol correspond to those set forth in JPL - PQ Semi-Annual, Document No. 900-675, September 30, 1974.

Isolated colonies of sporeformers were inoculated into 10 ml tubes of TSB. After incubation at 37°C for 72 hours, 1 ml of the broth culture was added to each of two 250 ml flasks containing 150 ml of synthetic sporulation medium (Lazzarini and Santangelo, J. Bacteriol. 94: 125-130). The flasks were then placed into a shaker - incubator at 37°C for 3-10 days until sporulation was 90% or greater as determined by phase contrast microscopy and spore staining. When adequate sporulation was observed, the suspension was filtered through sterile gauze to remove large pieces of precipitate. The filtrate was then centrifuged at 10,400 RCF for 20 minutes, followed by six distilled water washings and resuspension in 95% ethanol. Resuspension was facilitated using sterile glass beads and sonication (25 KHZ) for 12 minutes. Heat shock and non-heat shock titers were determined; stock cultures were stored at 4°C. Subsequent steps in the protocol again correspond to those in Document No. 900-675.

Table 2-E.1 shows the results to date of pure culture comparative tests between naturally occurring and MM'71 isolates. Assuming $P \leq 0.05$ to be significant, it can be seen that the two population types, in the spore or vegetative state, exhibit survival fractions that are not significantly different. While, of course, such an experiment does not conclusively pinpoint the nature of naturally occurring organism resistance, it does pave the way towards a

Table 2-E.1. Comparison of Survival Fractions of Pure Cultured
Naturally Occurring (N-O) and MM71 Isolates

SOLAR CONSTANT*, TEMP. (°C)	NONSPOREFORMERS			SPORES		
	N-O	'71	P**	N-O	'71	P**
0.1, -125°C	4.2×10^{-4}	3.5×10^{-5}	0.36	9.2×10^{-4}	3.0×10^{-3}	0.15
0.5, -15°C	1.4×10^{-4}	3.8×10^{-5}	0.12	7.2×10^{-4}	3.8×10^{-4}	0.06

*DOSE = 8×10^{3} ergs mm^{-2} (200-270 mm)

**PROBABILITY BASED ON t - DISTRIBUTION TEST

better understanding of it. These tests of naturally occurring microbes yield survival fractions for the nonshielded state; i.e., nonviable particulates were not present to protect cells from lethal spectra.

The question remaining in this task area is the following: "Is the major factor in naturally occurring organism resistance to SER the innate properties of these populations or the nonviable particulate shielding afforded them as they occur in fallout?" In an attempt to answer this question experiments have been designed to add particulate contamination to pure cultured naturally occurring isolates. The particulate contamination will be of a type and distribution representative of that present in natural fallout. If adequate resolution can be obtained, the influence of such particulate contamination on naturally occurring organism resistance should be defined. Complete reversion of the pure cultured isolates to the survival fractions noted for natural fallout would indicate particulate shielding to be the determinant of resistance, while no or slight enhancement of survival would point to the importance of the innate properties of naturally occurring cells. Quantification of particulate shielding effects would provide data on the impact of different levels of spacecraft cleanliness as they impact the effect of SER on resident microbial populations.

2.5.4   Future Activities

Efforts will continue to define the nature of naturally occurring population resistance to SER.

2.5.5   Presentations

Wardle, M.D. "Effect of Solar Electromagnetic Radiation on Micro-organisms," presented at the NASA Spacecraft Sterilization Technology Seminar, Cocoa Beach, Florida, August 1975.

SECTION III

POST LAUNCH RECONTAMINATION STUDIES
(NASA No. 193-58-62-03)

Contents   <u>Title and Related Personnel</u>

Cognizance: J. Barengoltz

Associate
Personnel: I. Ickovits (Bionetics)

3.1   POST LAUNCH RECONTAMINATION STUDIES

3.1.1   Introduction

The objective of the task is the development of an analytical technique for the evaluation of the probability of the relocation of particles from non-sterile to sterile areas on a spacecraft. The recontamination process is important for all multiple missions with separate microbiological burden allocations for various major spacecraft systems, and critical for life detection experiments that risk contamination from nonsterile components.

The approach has been to study the effects of typical mission environments on the redistribution of particles on spacecraft surfaces both analytically and experimentally. This study consists of three logical components, which have been reflected in the effort: (1) particle adhesion, (2) dynamic release mechanisms, and (3) particle transport. The effort in particle adhesion has been principally a particle release experiment, together with analytical work and attempts to correlate other data found in the literature and elsewhere. Under dynamic release mechanisms, meteroid impact and pyro firing have been modeled. The particle transport activity is an analytical effort which includes the development of codes for spacecraft geometry and orientation, forces acting on released particles, and trajectory.

All of these components are then assembled into an operational, integrated computer code which provides estimates for a particular mission type (e.g., Ref. 1).

Recently the task has been redirected to address the recontamination hazard for shuttle (STS) - launched planetary spacecraft. In this case the relocation of interest is transfer from the orbiter to the spacecraft prior to or immediately after separation. In addition to significant new work in the three areas discussed above, a new component (4) is being considered, namely the creation of new particulates (as opposed to pre-existing surface contamination).

3.1.2   Significant Accomplishments

3.1.2.1 Particle Adhesion.   An effort has been initiated to augment the current particle adhesion model. An experimental activity to obtain data for

a direct comparison of adhesion at atmospheric pressure, partial vacuum (~10 mm Hg), and high vacuum (16^{-6} mm Hg) has been started.

The first phase includes five size ranges of glass beads analogous to previous work at high vacuum (Ref. 2) at accelerations ranging from 100 gee to 30 kilogee*. The atmospheric pressure and high vacuum runs are performed simultaneously in a centrifuge. The evacuated centrifuge tubes are obtained through the use of a fixture shown in Figure 3-1 in conjunction with an oil diffusion pump vacuum system. A schematic of the sample in the test configuration in the centrifuge is presented in Figure 3-2. The particles are counted prior to and after testing by microphotography (Figure 3-3). A more detailed description of the procedures may be found in Ref. 3.

The first phase is about half completed at this writing. A second phase will consist of tests with dust of a known size distribution at all three levels.

3.1.2.2 Dynamic Release Mechanisms. No significant effort has been expended in this area during the reporting period.
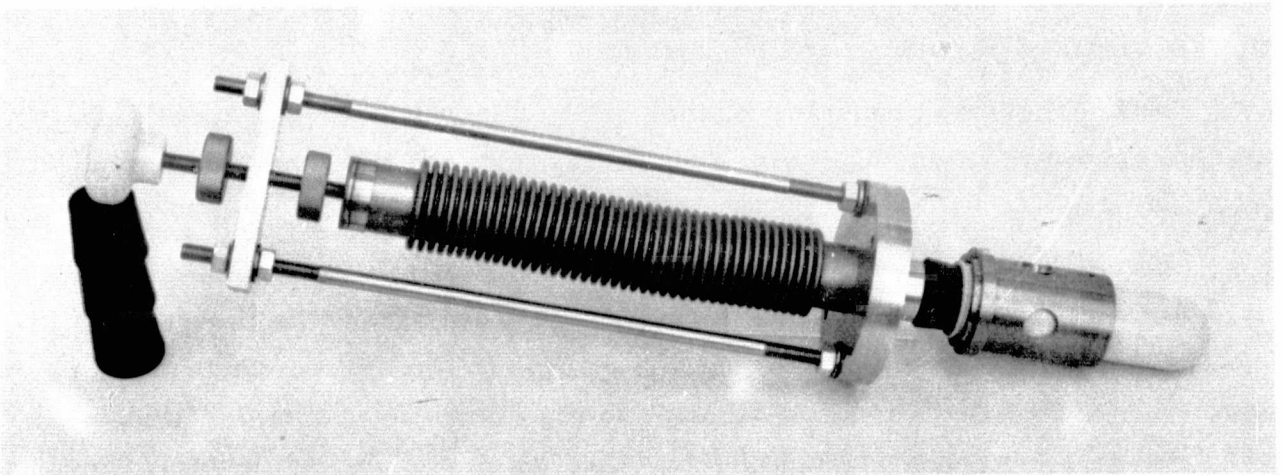
Fig. 3-1. Device to Accomplish Sealing of Sample Container Through Wall of Vacuum Chamber

*1 gee is the acceleration due to gravity at the surface of the Earth (9.8 ms^{-2}).
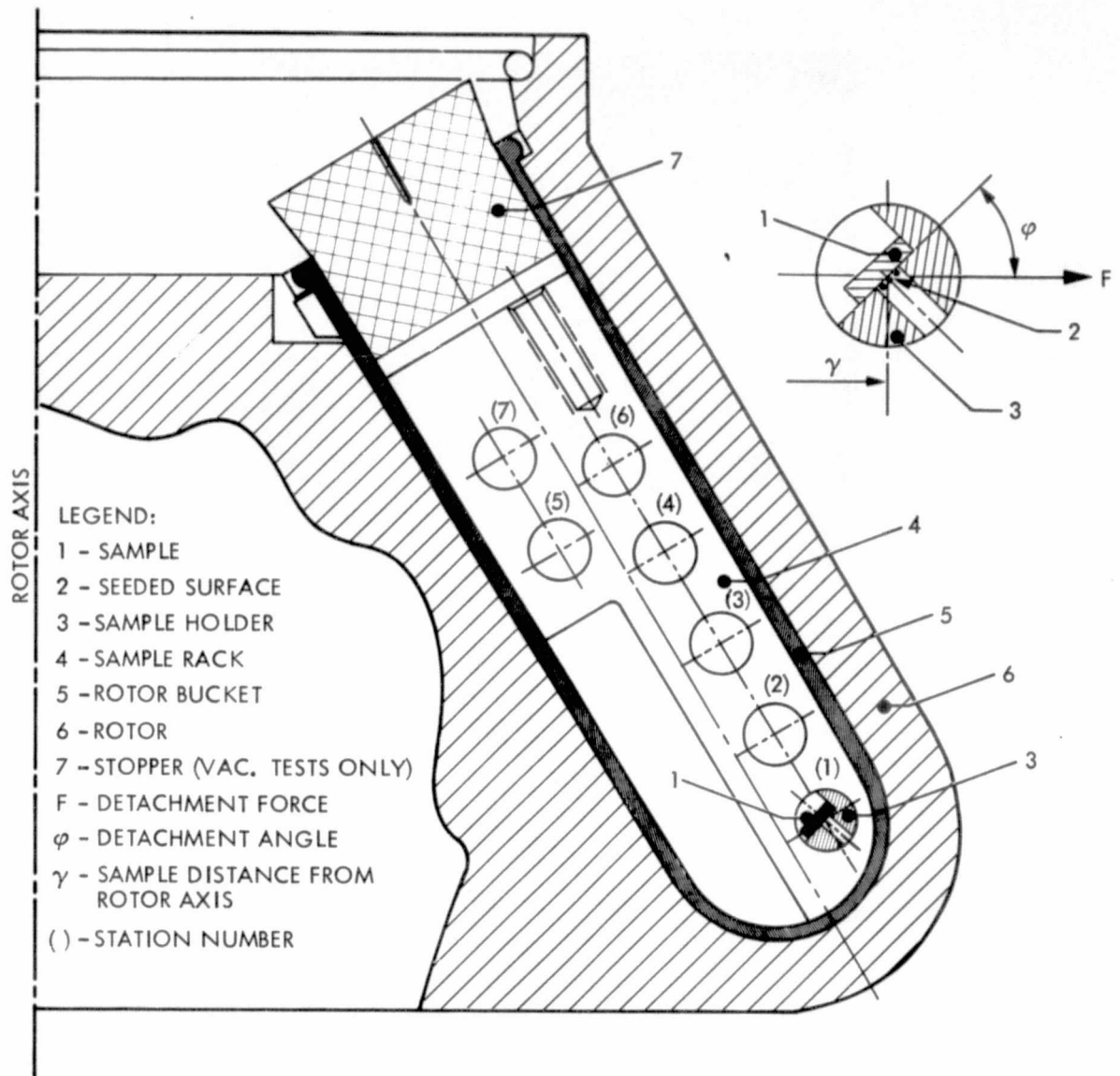
Fig. 3-2. Sample Installation and Nomenclature

3.1.2.3 <u>Particle Transport.</u>   During this reporting period, an area of major specificity to the STS orbiter was studied, its geometry.   In particular the geometry critically affects the electric field, which in combination with the electric charges residing on free particulates represents a major force.

The analysis of the electric field for an ellipsoidal geometry with variable axis dimensions was developed.   This physical model with the addition of a few planar pieces for the wings and tail was chosen to simulate the actual orbiter geometry.   The Debye screening due to the solar wind and photoelectron
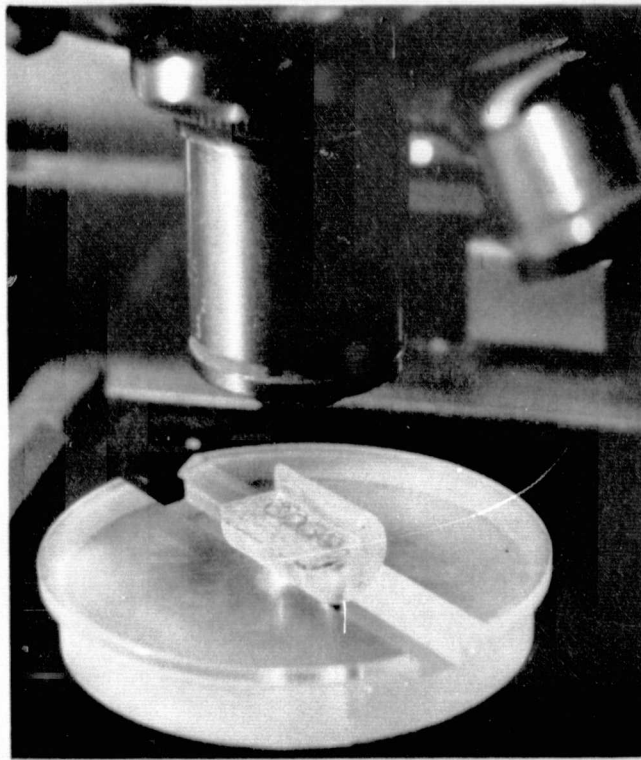
Fig. 3-3. Sample Holder Positioned for Counting
Under 100 × Microscope

plasmas has also been incorporated into the analysis. The resulting model
conforms to the earlier parametrizations. The local equilibrium charges and
effective average surface charge, required parameters within the electric
field computation, are found by the use of a previous analysis.

As part of the investigation into other forces affecting particle trans-
port, an analysis of the atmospheric drag force was completed. Additionally,
some of the rotational inertial effects on particle motion have been studied,
and their effects have been found to be non-significant. These new contri-
butions have now been computer encoded, debugged and are fully operational.

3.1.2.4 Sources of New Particles. During this reporting period, several
sources of new free particulates which could be more important than the
dynamic release of surface contaminant particles were considered. This
change in approach is due to the unusual STS orbiter surface materials which
may attenuate meteoroid impact and pyrotechnic device firing shocks very

efficiently. The analysis of these dynamic release processes will be accomplished with the existing model and suitable new material constants. The new particle processes under consideration are back splash from meteroid impact (the crater and the meteoroid material), flaking from edges and surfaces due to thermal gradients and wear, and the abrasion of aluminum - aluminum moving interfaces (e.g. separation joints and deployment hinges).

Samples of the principal orbiter surface materials have been obtained: high temperature, low temperature, and flexible reusable surface insulation. Detailed information on the physical properties and environmental test data are currently being sought.

3.1.3   Future Activities

The particle adhesion experiment will be completed. The data as a function of vacuum level will, through analysis, lead to a model suitable for STS ascent as well as on orbit phases. The dust data compared to the glass bead data will place some limits on material dependence for the first time.

The effort in particle transport will represent the major continuing activity in this task. All codes that depend on geometry will be revised, co-ordinate system transformation programs to include the orbiter's Earth orbit will be written, and the particle charging model will be updated to include the effect of the Van Allen belts. In addition, the transient electric field of the orbiter during terminator transit will be considered.

The activity in new particle sources will continue to the point of estimates of the number, size distribution and velocity distribution of the particles created.

3.1.4   Presentations

Barengoltz, J., "Post Launch Recontamination Studies," presented at NASA Spacecraft Sterilization Technology Seminar, Cocoa Beach, Florida, August, 1975.

3.1.5 References

1. Barengoltz, J. and Edgars, D., "The Relocation of Particulate Contamination during Spaceflight," NASA TM 33-737. Jet Propulsion Laboratory, Pasadena, Calif., 1975.

2. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 July - December 1973, JPL Doc. 900-655, p. 3-1, 1974.

3. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January - 30 June 1975, JPL Doc. 900-715, p. 4-13 ff., 1975.

SECTION IV

SPACECRAFT CLEANING AND DECONTAMINATION TECHNIQUES
(NASA No. 193-58-63-02)

Contents	Title and Related Personnel
Subtask A para. 4.1	**IMPROVED BIOSAMPLING TECHNOLOGY** Cognizance: R. Koukol Associate Personnel: W. Neiderheiser (Bionetics)
Subtask B para. 4.2	**EVALUATION OF VACUUM/HEAT STERILIZATION** Cognizance: A. Irons Associate Personnel: J. Barengoltz A. Ferreira (Bionetics) M. Peelgren
Subtask C para. 4.3	**CLEAN TRANSFER SYSTEMS** Cognizance: A. Irons Associate Personnel: M. Peelgren E. Cuddihy
Subtask D para. 4.4	**ADVANCED BIODETECTION METHODS** Cognizance: J. Jacobs Associate Personnel: L. Goforth F. Morelli (Bionetics)

4.1   IMPROVED BIOSAMPLING TECHNOLOGY

4.1.1   Subtask A Introduction

The objective of this task is to develop an improved biosampling technique for use on spacecraft hardware. Experience in past flight projects (Viking, Mariner 9, Mariner 6 and 7) indicates that a faster and more efficient sampling system would be beneficial to a flight project. The currently used swab-rinse technique allows data reproducability, however it requires approximately 8 hours of spacecraft time for each bioassy to be performed. Additionally the swab-rinse technique has been found to be only 30% to 50% efficient in recovering microorganisms.

Previous work in the Physical Removal of Spacecraft Microbial Burden subtask indicates that a jet deflection technique utilizing a liquid under pressure is very efficient at removing particulates. As shown in Table 4-A.1, Oscillating Rod Particulate Data, the removal efficiency was extremely high for all particle size ranges at three different GN_2 nozzle pressures.

The jet deflection technique produces an oscillation over a variety of nozzle pressures. It was found that utilizing this oscillation in conjunction with a liquid dispensed through a hollow rod produced very efficient particulate removal. Maximum efficiency with this system was obtained when the nozzle jet would pass through the rod axis where it intersected the oblique plane of the open end. This imparts a rotating fan of liquid on the surface which instantly flushes particles into the vacuum system.

4.1.2   Approach

This study is designed to examine the jet deflection technique as a removal system and determine its feasibility and application as a spacecraft hardware biosampling device.

Activities involved in this study include the determination of potential sampling liquids and development of a collection device. The selection of sampling liquids is critical to the application of the system for bioremoval and detection. Acceptable liquids must be completely compatible with spacecraft hardware to eliminate any possibility of damage.

Table 4-A.1. Oscillating Rod Particulate Data

COUNTS FROM 1/2 x 1/2 mm CONTROL SURFACE	PARTICLE SIZE, μm								GN$_2$ NOZZLE PRESSURE psig
	3 – 5		5 – 10		10 – 25		> 25		
ORIGINAL	53	43	45	53	112	72	6	12	----
VACUUM	53	43	38	41	60	60	2	6	----
SPRAY	0	3	0	0	0	0	0	0	0.5
ORIGINAL	76	41	40	73	63	106	7	11	----
VACUUM	76	41	40	69	44	97	1	11	----
SPRAY	0	0	0	0	0	0	0	0	1.0
ORIGINAL	62	72	162	102	182	112	13	17	----
VACUUM	62	72	100	80	70	50	0	0	----
SPRAY	0	0	0	0	0	0	0	0	3.0

GM # 1543637 STANDARD DUST
FOGGED OPTICAL GLASS
NEEDLE STANDOFF — 1 mm FROM BLOW NOZZLE
2.8 mm PRESSURE VACUUM NOZZLE

The collection device must employ a technique which traps the micro-organisms without the dessication normally associated with vacuum bio-sampling devices.

4.1.3   Significant Accomplishments

To achieve nominal sampling efficiency, significant effort has been spent on tuning the oscillating rod sampling device. Included in this effort is determining the oscillating rod (hypodermic needle) angle, GN$_2$ blow angle and pressure, as well as the optimum distances between these functions, the vacuum nozzle, and the sample.

Initial biological testing has examined the sampling efficiency of the oscillating rod system using several population levels of <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u>. The removal efficiency has ranged from 51% to 83%.

4.1.4   Future Activities

The bioremoval efficiency determination for several systems employing the jet deflection technique will be completed. A recovery system will then be developed which will allow the direct detection of viable organisms. The use of the recovery system will allow for the determination of the microorganism recovery efficiency. Finally, liquids acceptable to the spacecraft hardware engineers and compatible with bioassay techniques will be determined.

4.1.5   Presentations

None.

4.2 EVALUATION OF VACUUM/HEAT STERILIZATION

4.2.1 Subtask B Introduction

Vacuum/dry heat sterilization studies were conducted at JPL and reported on in detail in a previous JPL Semi-Annual Review (Ref. 1). These studies indicated the existence of a vacuum/heat synergism which reduced the dry heat resistance of <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u> (BSN), spores relative to their resistance at ambient pressure. One of the few previous research efforts that specifically studied the difference in dry heat resistance of bacterial spores in a vacuum and at atmospheric pressure was the reported work of Davis, Silverman, and Keller (Ref. 2). They reported better survival of <u>Bacillus</u> <u>subtilis</u> var. <u>niger</u> spores at atmospheric pressure than in a vacuum of 10^{-8} to 10^{-10} torr at both 60 and 90°C.

If a true vacuum heat synergism exists which lowers the dry heat resistance of bacterial spores, then this technique, or procedure, could be utilized to sterilize spacecraft components that are heat labile. An additional advantage of this procedure would be that a lower temperature for sterilization could increase the reliability of components.

As a result of the information gained from JPL's studies it was theorized that the usual characterization of the thermal death of microorganisms in terms of length of exposure to a particular temperature requires modification. It was further theorized that the total thermal flux incident on the specimen must be considered to properly understand thermal death. The hypothesis is that the internal temperature of an organism, rather than the temperature of the surface on which it is resident (substrate), is the correct parameter. The organisms ability to withstand a given substrate temperature appears to depend on the chamber material and color (infrared reflectivity) and the chamber wall temperature as well (Figure 4-B.1). Thus, it has been postulated that the internal temperature of the spore is a function of conduction from the substrate, thermal radiation from the substrate reflected by the chamber wall, thermal radiation by the chamber wall and thermal radiation by the spore itself.

The primary objectives of this task are to elucidate and define the effects of chamber wall temperature, infrared reflectivity, and other
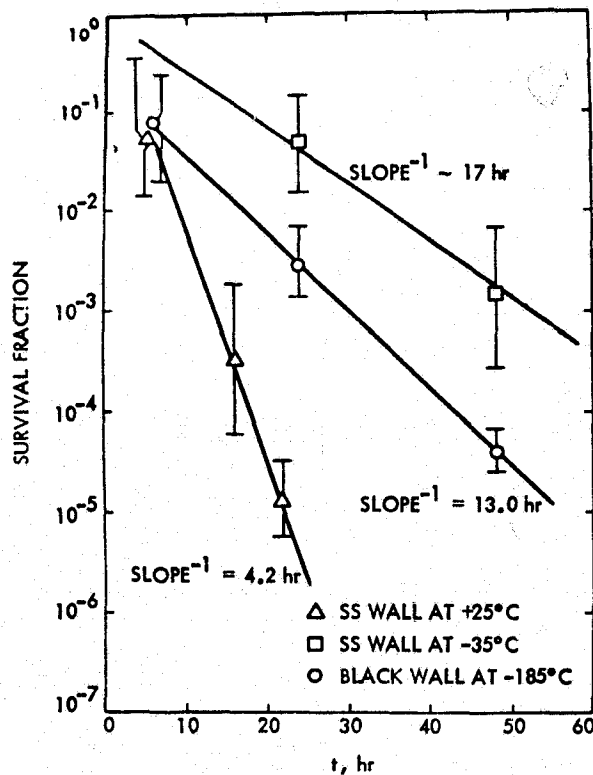
Fig. 4-B.1. _Bacillus_ _subtilis_ var. _niger_ Spores in Vacuum $T_o = 80°C$

parameters on the sterilizing efficiency of dry heat concurrently applied with vacuum or atmospheric pressure as well as to develop a better understanding of the mechanism of dry heat kill and the vacuum-heat synergism which decreases the heat resistance of spores.

4.2.2 Approach

Further vacuum heat studies will be conducted in a unique system, with good controls and geometry, which will lend itself to meaningful data analysis. The analysis will permit quantification of the various components of thermal flux and microbial internal temperature, and allow the thermal inactivation data obtained to be modeled.

4.2.3 Significant Accomplishments

A fixture has been fabricated which will permit measurement of the parameters influencing the temperature of microbes resident on surfaces

while exposed to vacuum and heat. The fixture will permit exposure of organisms to various chamber wall temperatures, substrate temperatures, chamber wall reflectivities and exposure durations, while under a vacuum of approximately 1×10^{-5} torr. The fixture, Figure 4-B.2, consists of a polished aluminum plate with milled out slots, drilled to hold square "stages" which will be inoculated with test organisms. This plate has electric heater strips bonded to the back surface opposite the "stages." Thermocouples are attached to the back surfaces as well as through the shaft of some of the "stages" to a point just under their surfaces to permit control of substrate temperature and measurement of "stage" surface temperature. Facing the "stage" plate, and at a distance of 1 inch, is an aluminum heat exchanger plate containing 12 flow through channels for gas or liquid. This plate, which appears as the chamber wall to the test organism, is polished on one side and painted flat black on the other. Six thermocouples are located under each surface at various locations to permit measurement of surface temperatures. The
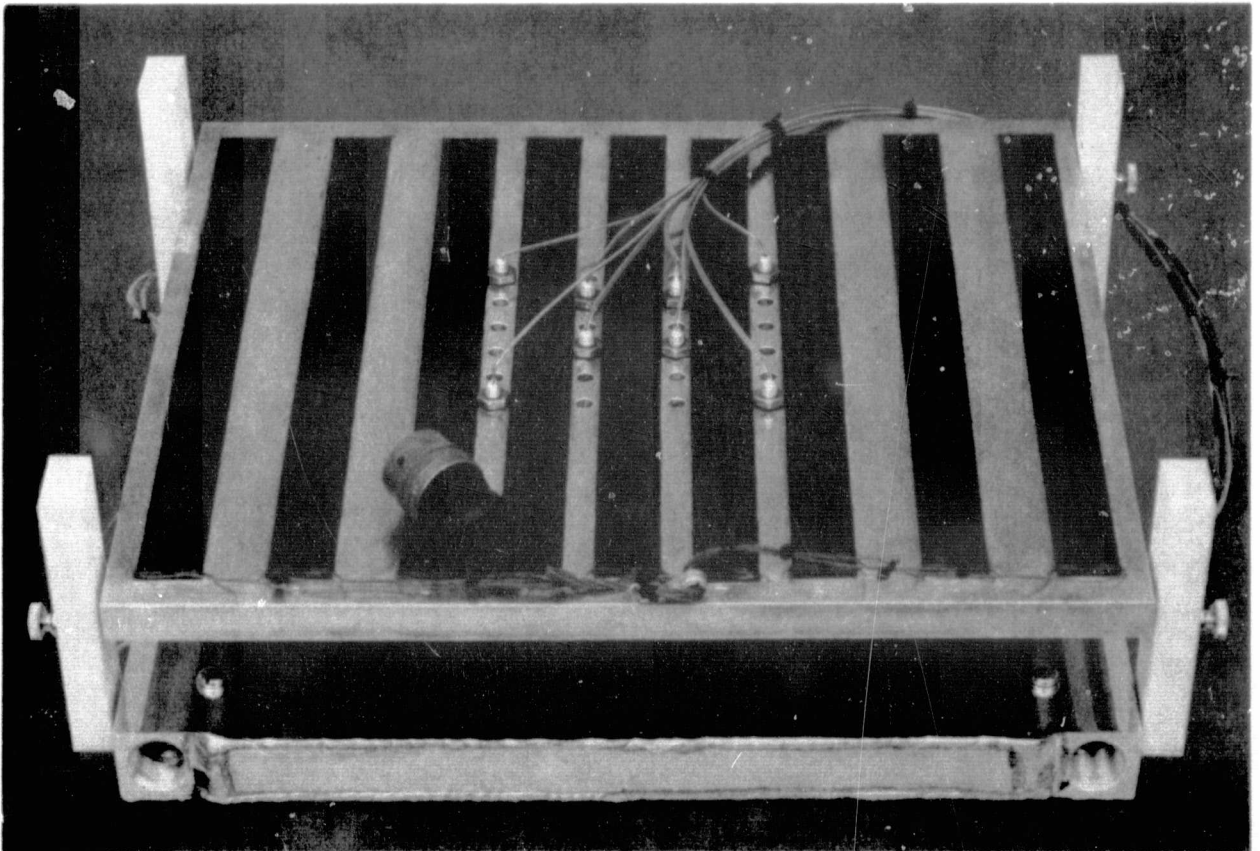
Fig. 4-B.2. Vacuum/Heat Test Fixture

heat exchanger plate can be heated with gas beyond 100°C or cooled with liquid N_2 to -185°C to meet the required test conditions. Additionally, the plate can be reversed to present either a black or polished surface to the inoculated "stages."

This fixture will be used in existing vacuum chambers, Figure 4-B.3, to produce the required test conditions regardless of the chamber wall color or temperature. Auxiliary heat exchangers utilizing heated or cooled gasses will also be used with this fixture configuration to augment the electric heater strips and permit reduction of the time to reach test temperature and return to ambient. This fixture configuration permits more precise measurement and control of chamber wall and substrate temperature, thermal radiation from the substrate reflected by the chamber wall, thermal radiation by the chamber wall and thermal radiation by the spore itself. These measurements will make possible the calculation of the internal temperature of organisms as compared to their substrate.
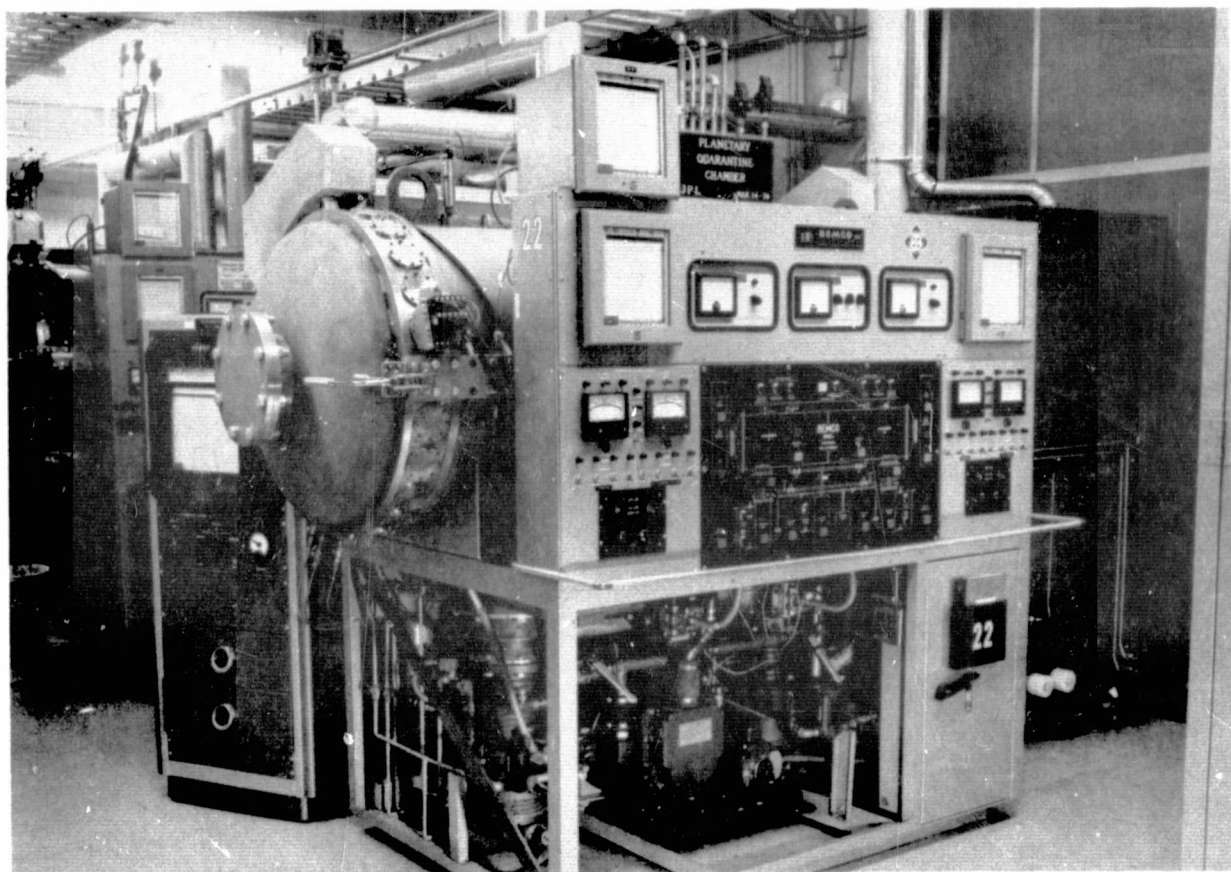
Fig. 4-B.3. Planetary Quarantine Vacuum Chamber

4.2.4 Future Activities

Spore cultures of B. subtilis and B. brevis (a so-called "hardy spore"), as well as naturally occuring organisms, will be exposed to vacuum and heat. The naturally occuring organisms will not be cultured prior to exposure. Instead they will be collected on fallout strips located in spacecraft assembly areas and exposed to the test conditions in their pristine state.

The cultured test organisms will be inoculated onto the polished aluminum stages or tacks located on the test fixture. After exposure to vacuum and heat under varying test conditions of exposure duration, chamber wall reflectivity, chamber wall temperature and substrate temperature, the tacks will be assayed for survivors. In the case of naturally occuring organisms, the test and assay conditions will be the same except that the organisms will remain on the fallout strips during exposure to the vacuum/heat and the strips will be assayed.

Survival fractions will permit elucidation and definition of the effects of the test conditions and other parameters on the sterilizing efficiency of dry heat concurrently applied with vacuum or atmospheric pressure.

4.2.5 Presentations

None.

4.2.6 References

1. Planetary Quarantine Semi-Annual Review, Supporting Research and Technology, 1 January - 30 June 1975, JPL Doc. 900-715, pp. 4-26 to 33, 1975.

2. Davis, N.S., Silverman, G.J., and Keller, W.H., "Combined Effects of Ultrahigh Vacuum and Temperature on the Viability of Some Spores and Soil Organisms," Appl. Microbial. Vol 11, pp. 202-210, 1963.

4.3   CLEAN TRANSFER SYSTEMS

4.3.1   Subtask C Introduction

The objective of this task is to develop a cleanable and sterilizable
seal configuration to be used as a required part of a clean or sterile transfer
system.   The transfer system may be required for repair of sterile spacecraft
prior to launch; on board a shuttle craft, for a sterile transfer of equipment or
materials from one facility to another, or for the placing of sample return con-
tainers or their contents into a biobarrier system.   Such a transfer system
must prevent microbial, particulates, atmospheric (gaseous) contamination of
materials or biobarrier systems during the transfer process.   When two
enclosures are mated, viable organisms can be trapped between the mated
surfaces and can be transferred into the sterile barrier when the separation is
made between the two surfaces.   In this study, a concept, as seen in
Figures 4-C.1, 4-C.2 and 4-C.3, is being investigated which utilizes a
multiple use, heat sealable, heat sterilizable transfer unit and seal system for
the enclosure interfaces that will prevent the transfer of organisms or
atmospheric contamination across this barrier-to-barrier interface.

4.3.2   Approach

In order to fulfill the objectives of this task a test program was con-
ducted to develop an understanding of the material, design, and sterilization
requirements of a sterilizable seal system.   Seal materials were evaluated
relative to their physical, chemical and biological properties at temperatures
ranging from 180 to 240°C.   These temperatures were chosen as a result of
previously obtained data which indicated that there was a high probability of
achieving sterility within a reasonable time span of 1 to 1.5 minutes at
temperatures in excess of 160°C.   This time/temperature takes into considera-
tion the time to reach temperature as well as the time required to return to
ambient.

It was decided that candidate thermal seal materials should be able
to adhere strongly and uniformly over the surface of another material which
would function as a heat source.   Upon heating, the seal material should soften
or melt to a specified depth along the interface at approximately the specified
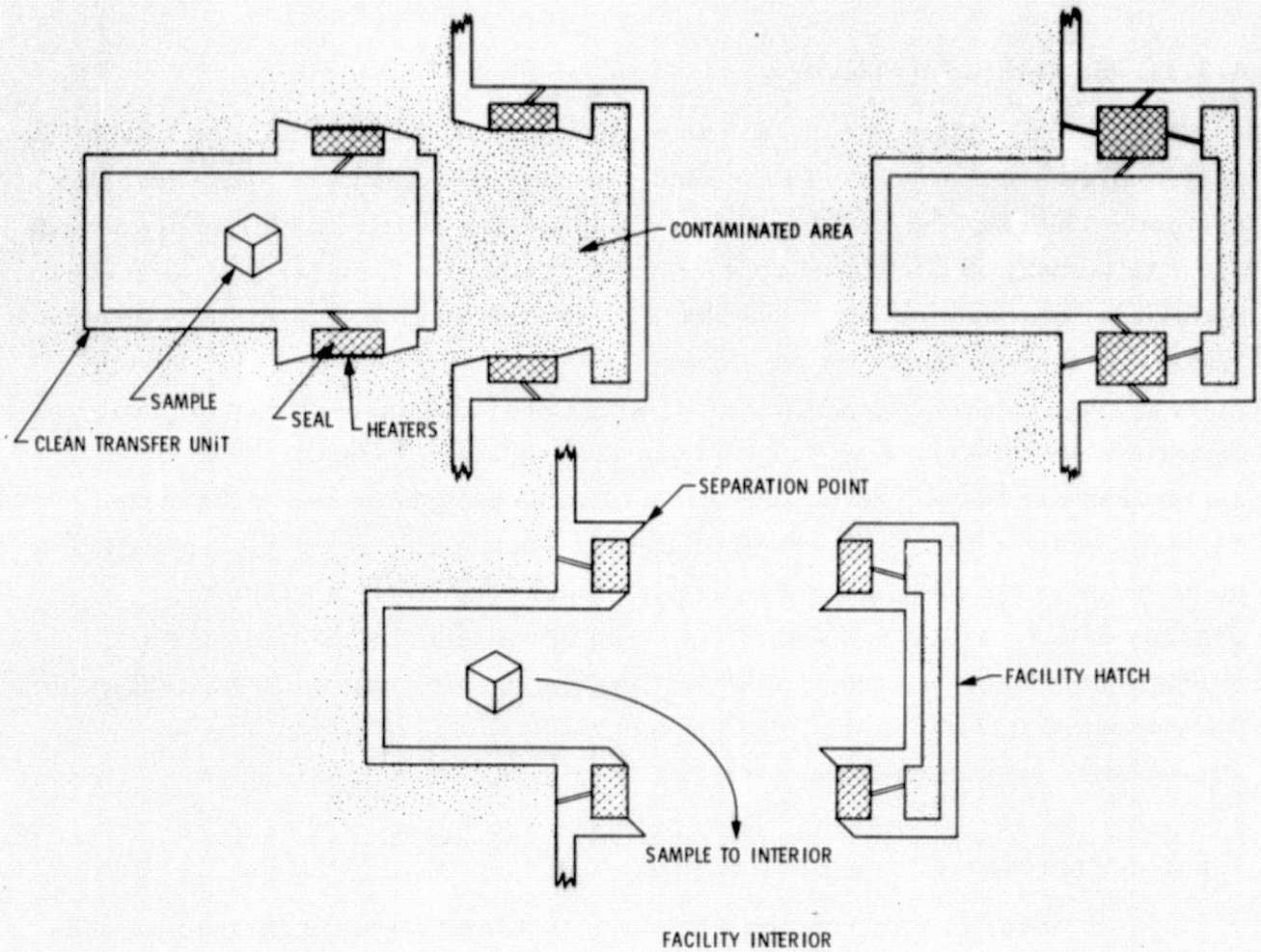
Fig. 4-C.1. Facility Interior, Figures of Transfer Process

temperature, such that de-adhesion would occur and the seal material could be clearly separated from the heat source. When the seal material was returned to press against the heated surface, it was to have the capability of reestablishing a strong and uniform adhesion when the system was cooled. Alternately, it was assumed that a screen or grid of heater wires could be embedded within the seal material. Upon heating the wires electrically, the seal material was to soften or melt along the plane of the heater wires, such that a clean separation could be achieved.
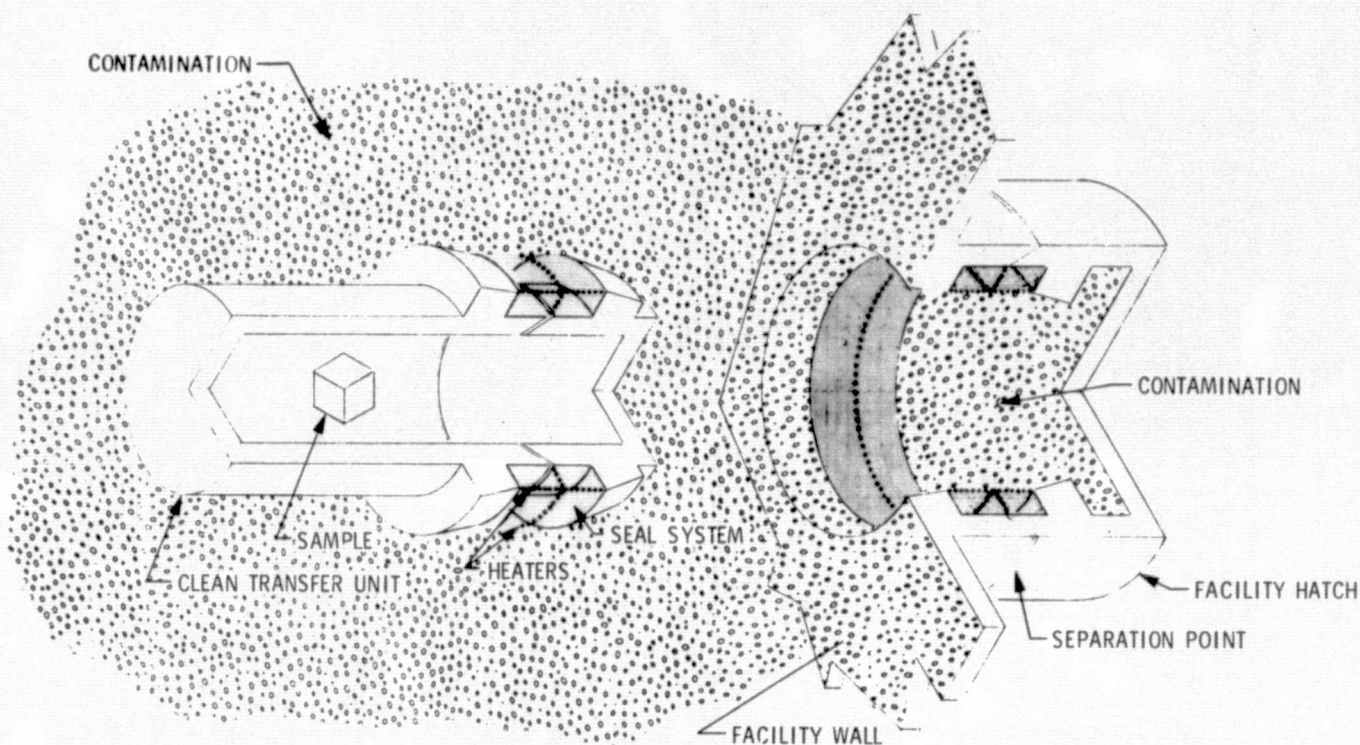
Fig. 4-C.2. Facility Interior, Figure of Unmated Enclosures

4.3.3   Significant Accomplishments

Several materials theoretically having the physical properties required
of the seal system were initially evaluated, and the following two general
classes of materials were identified as probable candidates:

1)   High temperature melting point crystalline waxes.

2)   Low molecular weight polymers having high temperature melting
      or softening points.

A fixture, as shown in Figure 4-C.4, was fabricated and is presently
being used for the evaluation of heat seal and cutting mechanisms as well as
for use with the candidate materials in order to determine their adhesion,
melting, penetration and thermal stability characteristics.

Waxes were considered to be excellent candidate materials because of
their inherent property of melting sharply to a non-tacky liquid phase which
would allow them to separate cleanly from a heated surface. However, waxes
as a general class melt at lower temperatures than required for this task. A
survey of commercial waxes identified only three having melting points high
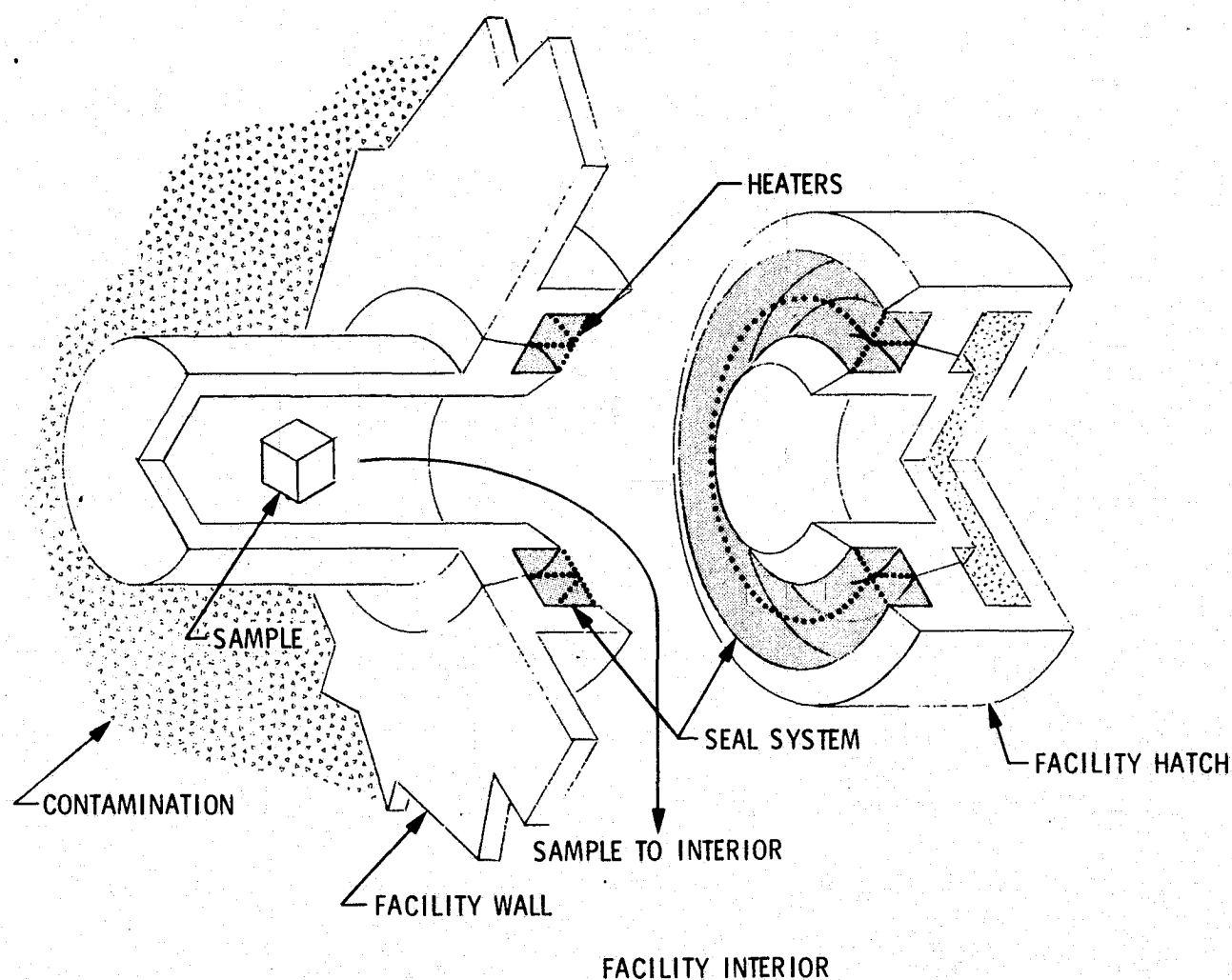
FACILITY INTERIOR

Fig. 4-C.3. Facility Interior, Figure of Mated Enclosures

enough to permit them to be considered as candidate materials for this program. Two of these have a melting point (M. P.) of 140°C and one has a M. P. of 240°C. The waxes can be blended to yield waxes having sharp melting points intermediate between 140 and 240°C.

The mechanical properties of waxes are poor and, as a class, they are usually very brittle. These three waxes are no exception. As part of this program, investigations were carried out to identify inert fillers which could be added to the waxes in order to improve their mechanical properties, without sacrificing melting characteristics. Generally, common discrete particle fillers such as glass beads, powders, etc., were found unacceptable. They typically resulted in a product which easily crumbled or fell apart. Fibrous
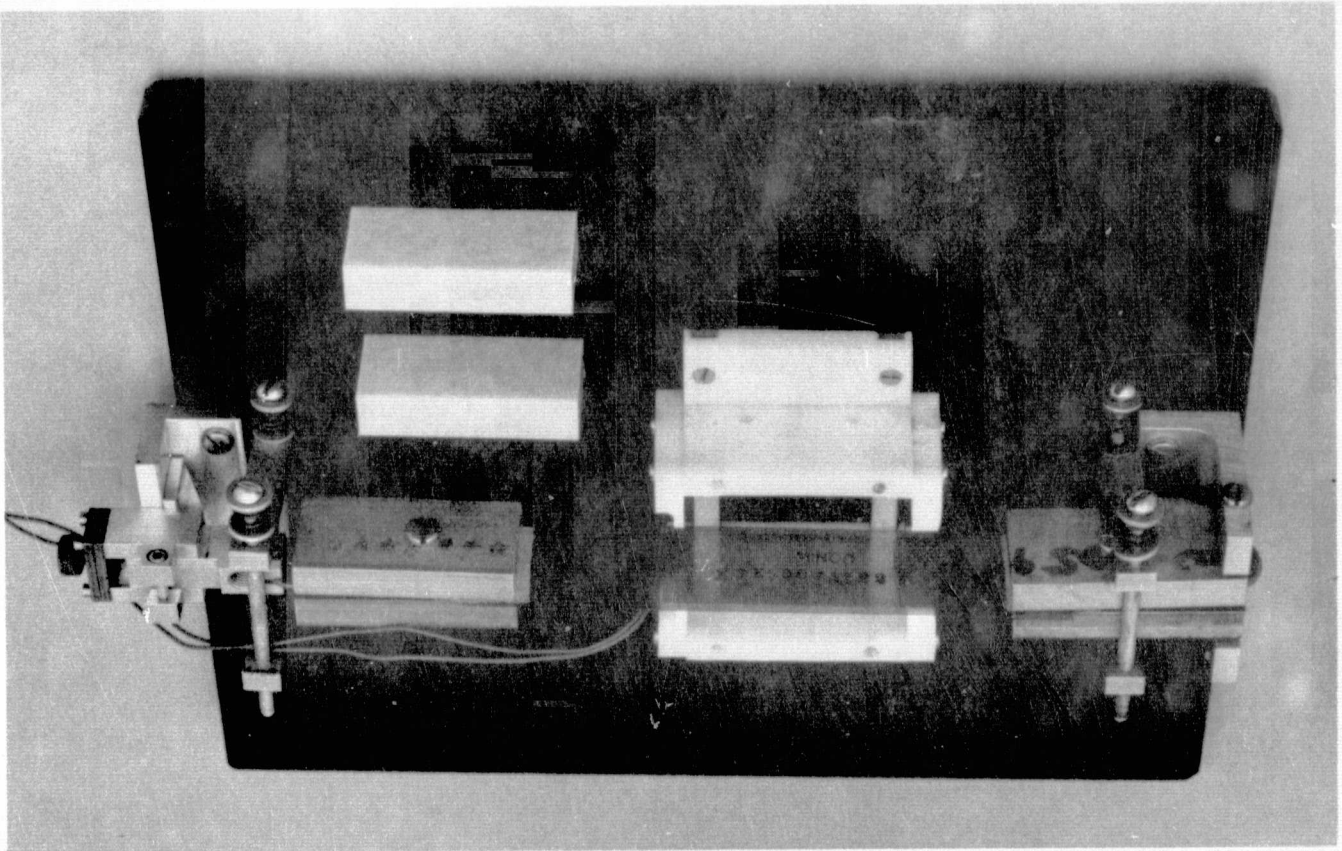
Fig. 4-C.4. Material Test Fixture

fillers were found to be much better, for they apparently functioned to hold the
wax together.

All softening and melt point tests were carried out using heater wires
encased in Kapton® film, and therefore, adhesion was judged relative to Kapton.
The following information has been gained as a result of studying waxes.

1) All three waxes when mixed with filler materials adhere strongly
   and uniformly to the heater strip upon heating but noticeably
   contract upon cooling and tend to disturb the heaters. Alternately
   they have a tendency to crack if the Kapton heater strip is
   mechanically restrained to prevent its distortion.

2) Crystalline waxes undergo a large volume change upon heating
   that tends to displace quantities of material and thus produce voids
   in the seal material upon cooling.

3) There is a great tendency for these waxes to outgas when heated to the predetermined sterilizing temperature range.

It was decided that waxes did not meet the seal requirements and that subsequent testing should be concentrated on polymers reportedly possessing the required properties.

Of the two general classes of polymers, i.e., thermosetting and thermoplastic, only thermoplastic polymers were considered able to meet the revised seal requirements, which were upgraded as a result of information gained from working with the crystalline waxes. These materials have the following required characteristics:

1) Thermal stability at 200°C (no outgassing or degradation).

2) Softening point near 200°C, not yielding a runny liquid phase, but sufficient softening to permit:

   a) Conformal coverage of seal material against a heated mating surface, and

   b) Easy penetration and permanent retention of any viable or non-viable particulates located at the seal interfaces when sealing occurs.

3) No adhesion to the mating surface when cooled to room temperature.

4) Capability of functioning for several heating cycles.

5) Solid but not brittle at ambient temperature and with some elastic qualities.

The high temperature requirement (ca. 200°C) severely limited the number of candidate materials. To date, three (3) promising classes of materials have been identified. These are: polyester elastomers, fluorinated elastomers and silicone polycarbonate block copolymers.

4.3.4   Future Activities

Work will be continued to evaluate material as well as heat seal and cutting mechanisms. Successful testing will lead to development of a 2-plane test fixture to determine the feasibility of the transfer system.

4.3.5 Presentations

None.

4.4   ADVANCED BIODETECTION METHODS

4.4.1   Subtask D Introduction

The primary objective of this task is to develop new and more efficient techniques for the detection and enumeration of microbes on surfaces. Major emphases will focus on developing techniques which require no intimate contact, are direct measurements, provide a permanent record and produce real-time quantitative data. Such systems would prove invaluable as a basic research tool, possible assay of spacecraft hardware, and sample return analyses.

Several techniques will be examined, among which will be lasers, photoacoustic spectroscopy and the one that is presently being studied — a biodetection system utilizing macrophotography and multispectral analyses.

4.4.2   Approach

The technique coupling macrophotography and multispectral analyses is an attempt to convert technology which allows remote detection of biosystems on a macroscopic scale, i.e., multispectral sensing of agricultural and forest crops as well as marine populations, to allow the detection of microscopic biosystems such as microorganisms on a surface.

Preliminary activities involved the formulation and testing of macrophotographic specifications (films, light source, filters, exposure time and camera set up). Special emphases were put on examining the limitations of this system relative to the smallest size object that could be adequately photographed.

The results of the preliminary activities were used to develop a schematic outline of the biodetection system. The system consists of three main components — specimen, photography and image processing as shown in Figure 4-D.1. The system is explained in the following paragraphs.

Three specimen, Bacillus subtilis var. niger (spores and vegetative), glass Beads (5-10 microns) and Arizona dust (5-10 microns) were used in the initial testing. These specimen were deposited on coated aluminized glass slides scribed to provide a gridwork of 4 x 6 mm (approximately) rectangles.

SPECIMEN   PHOTOGRAPHY   IMAGE PROCESSING
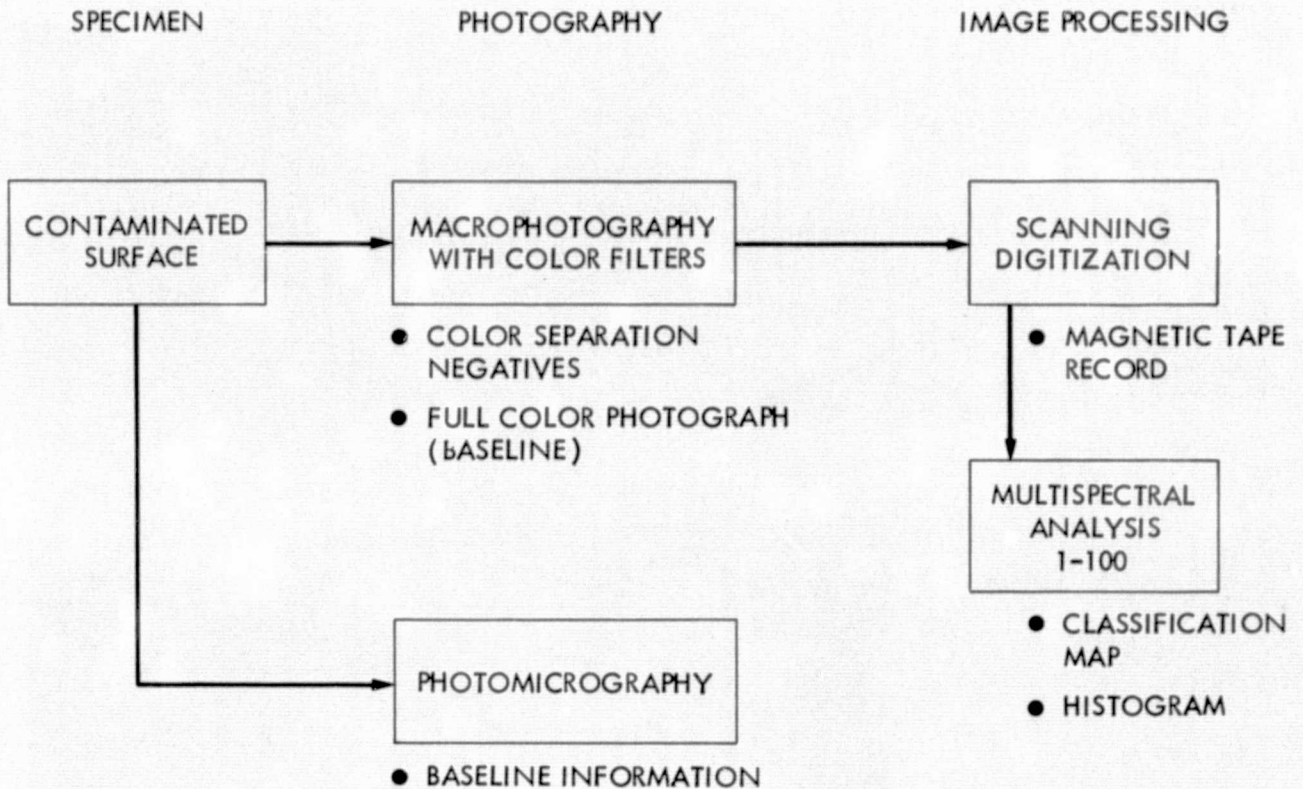
Fig. 4-D.1.   Biodetection System

Macrophotographs were made of each slide using a fabricated camera set-up (JPL Photolab) Figure 4-D.2 consisting of the following components: Polaroid MP-3 copy stand (1), Aristo light box (2), Nikon 35 mm SLR camera with long bellows extension and f/3.5 macro lens mounted in reverse position for a 5× photomagnification factor (3), Kodak 5069 mm hi-contrast copy B/W (black-and-white) film was used for colar separation photographs. Wratten filters used were: neutral 1.9 ND (Neutral Density), blue 1.0 ND + 47B, green 1.3 ND + 55, and red 0 ND + 29. A color reference picture of each slide was taken using Kodacolor II 35 mm color film.

Photomicrographs (100× and 500×) of each specimen were taken using a Leitz microscope. The photomicrographs are to be used as baseline information.

Data processing consists of scanning and multispectral analysis. Each B/W 35 mm color separation film frame for a given specimen was scanned on a Perkin-Elmer PDS microdensitometer, digitized and recorded on magnetic tape.
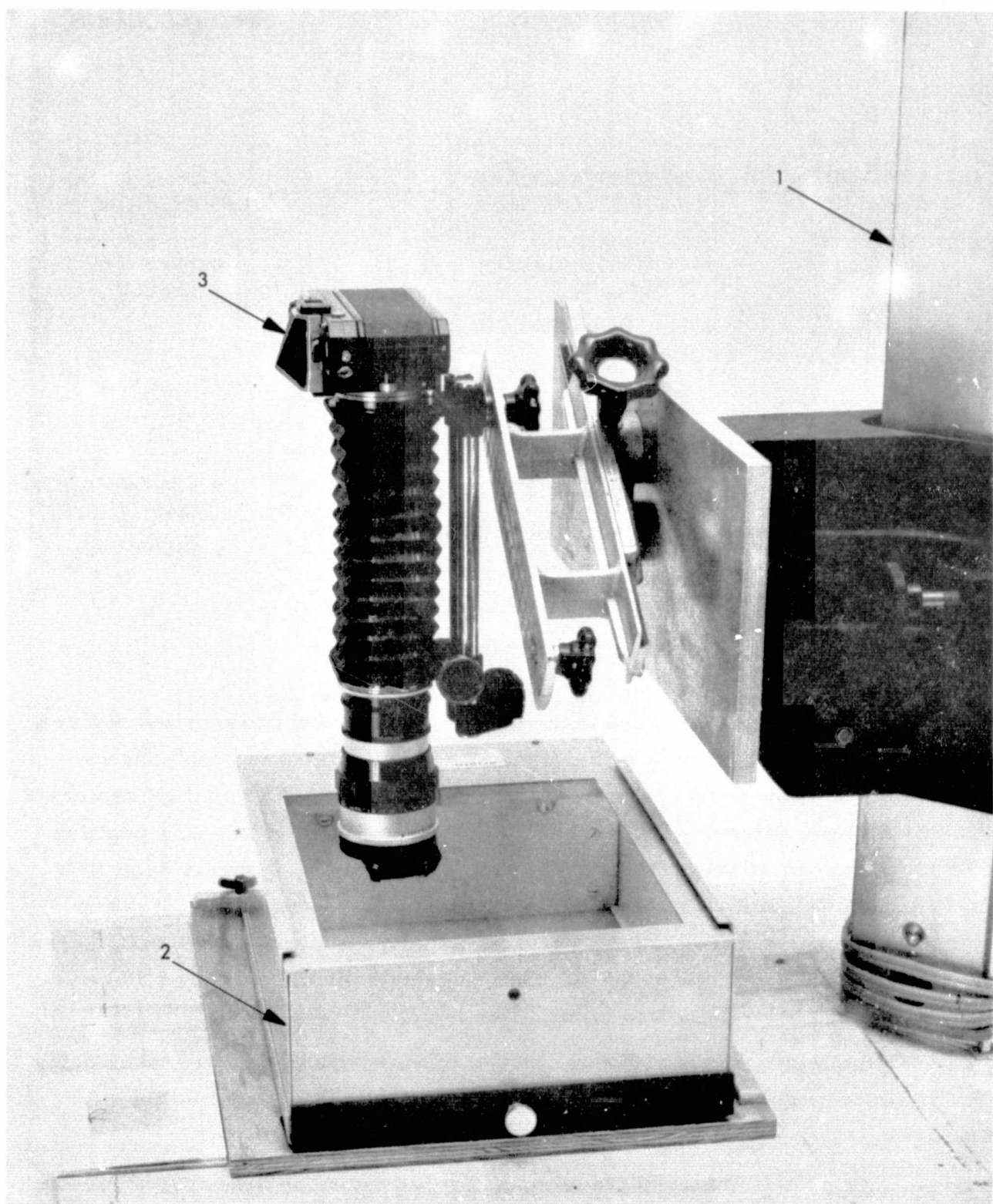
Fig. 4-D.2. Biodetection Macrophotographic Camera Jig

Each set of digital color separation images was analyzed on the GE Image-100 (I 100) system Figure 4-D.3, to determine the existence and degree of a multispectral signature associated with any of the specimen.

4.4.3 Significant Accomplishments

During this feasibility period preliminary photographic specifications were tested, and the data processing, scanning and multispectral analysis procedures were formulated.

4.4.3.1 Macrophotography. With reference to the first objective to determine the size of the smallest objects that can be detected using macrophotography, it has been demonstrated that objects as small as 2 microns can be detected using a camera and macro lens stopped to f/8. At this setting, however, the macrophotographic film image of any object less than about 12 microns in diameter appears as an Airy disk of 12 micron diameter due to the diffraction limit of the optics. At f/3.5 (wide open) the Airy size limitation should be reduced to about 6 microns. The effect this has on feature class area percentages will be evaluated.

4.4.3.2 Multispectral Analyses. It has been demonstrated that organisms can be detected or distinguished by characterizing spectral signatures as shown in Figure 4-D.4.

4.4.4 Future Activities

Immediate attention will be given to modifying the camera set-up to provide more stability to the system.

Spectral analyses to characterize a variety of organisms and particulates will be done to develop a data bank, which will be used as a basis for formulating contamination assays and other research applications.

4.4.5 Presentations

None.

STANDARD SYSTEM
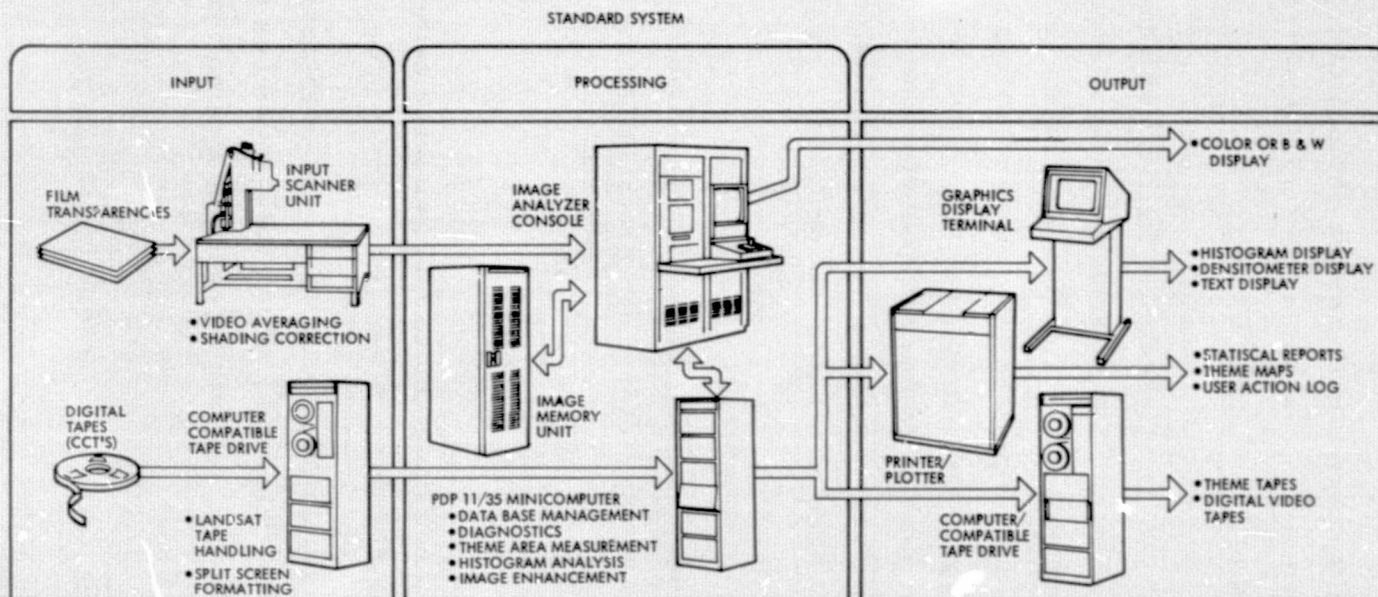
Fig. 4-E.2   Image-100 system configuration

REF MARK

SHADOWS

BACKGROUND

MICROCOCCUS

MICRODETECTION
EXPERIMENT MCI
AREA 2
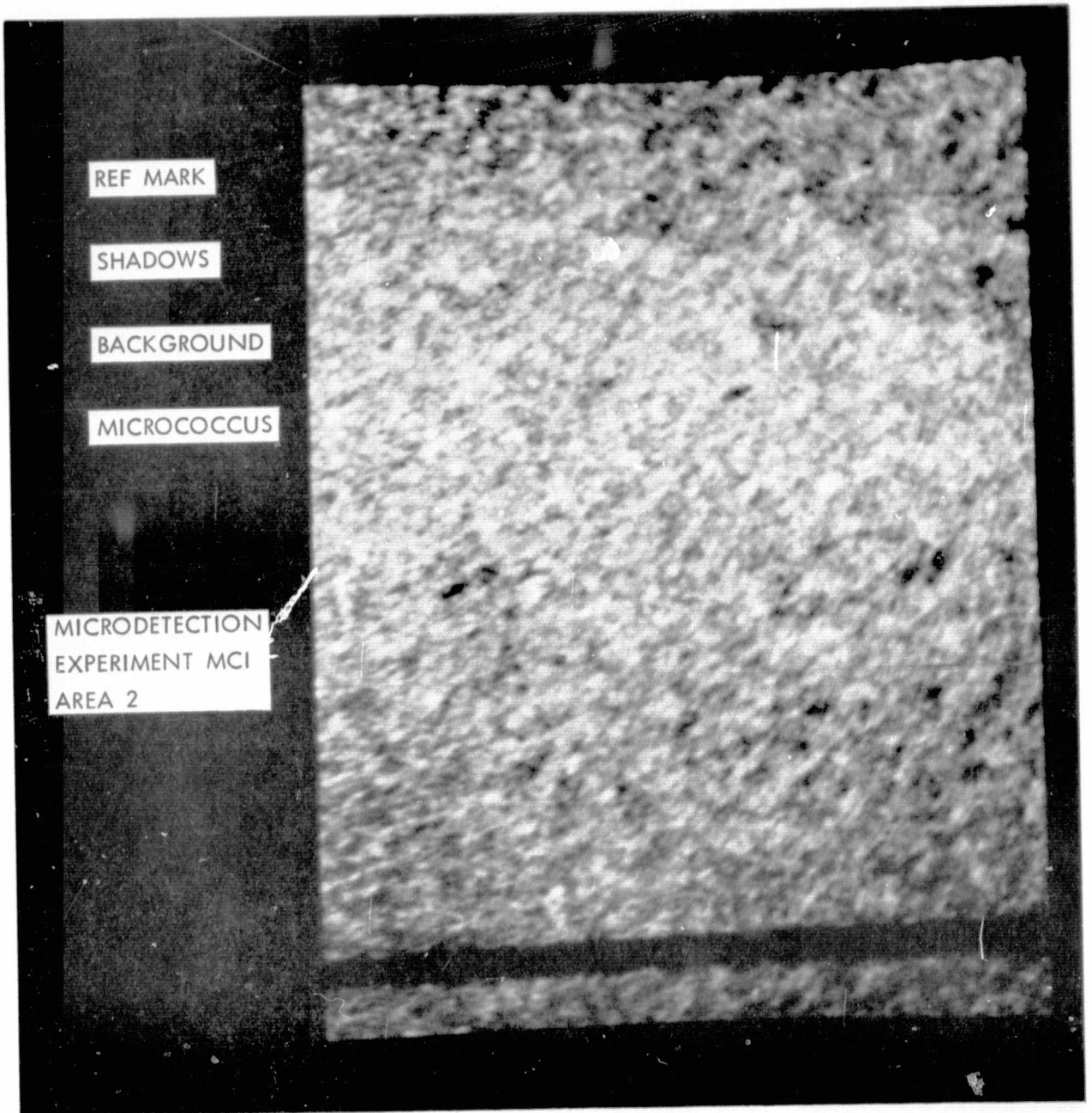
Fig. 4-D.4   Spectral Analysis of Organisms

SECTION V

PLANETARY QUARANTINE CONSIDERATIONS
FOR
SHUTTLE LAUNCHED SPACECRAFT
(NASA No. 193-58-63-07)

5.1 PLANETARY QUARANTINE CONSIDERATIONS FOR SHUTTLE LAUNCHED SPACECRAFT

5.1.1 Introduction

The objectives of this task are to assess the affect of Shuttle Transportation System (STS) ground, launch, on-orbit and abort/return operations on planetary spacecraft (S/C) to determine if they impact quarantine program requirements.

5.1.2 Significant Accomplishments

During the report period, two activities were completed. The first dealt with obtaining a better understanding of the previously identified impact areas (JPL 900-715, October 1, 1975) and the nature and degree of the potential problem. The results of this evaluation indicate that present STS operational plans (baseline and alternatives) continue to pose potential problems for PQ.

The second activity consisted of reviewing the proposed STS payload environments to identify potential contamination sources. The review included ground, launch, on-orbit and abort/reentry operations.

5.1.2.1 STS/PQ Impact Assessment. For the purposes of this study, impact was defined as either Type 1 — the S/C encountering contamination of the nature or degree that could potentially require reprocessing of the vehicle to reduce microbial or particulate levels; Type 2 — implementation of quarantine operations that might impact the STS Program Office constraint that the STS turnaround time not exceed 160 hours. Basically, this constraint means that all on-line S/C-orbiter integration operations must occur within 160 hours[*] from the time the orbiter lands, culminating one mission, until it is launched on a planetary mission. This requirement assumes that there will be no explainable interface anomalies once the S/C enters the STS 160-hour timeline. Since quarantine operations have not been considered within that 160 hours, any on-line requirements, for quarantine purposes, would automatically impact the timeline; or Type 3 — either contamination or timeline impact associated with contingency or on-orbit operational planning.

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[*]This assumes two weeks (5 days/week, 16 hours/day).

A brief description of the impact areas, designated impact type and the nature and degree of the potential problem are presented below.

1. Provision for Spacecraft Environmental Control (Type 1). Following the installation of the spacecraft in the Orbiter Processing Facility (OPF), there is no provision for power or continuous environmental control in the orbiter cargo bay from closeout through mating of the orbiter with the external tanks in the Vehicle Assembly Building (VAB). The time from rollout until power on is approximately 20 hours. Purge capability will be provided, although flow patterns are unknown. Purge will not be available during the stacking operation, a period of approximately 12 hours. During this interim, the S/C has a good probability of being exposed to the VAB environment which is > Class 100,000. The orbiter payload bay doors and vents will be closed, however, thus affording the payload some protection.

2. On-Pad Installation of RTG (Radioisotope Thermoelectric Generators (Type 2 and 3). Almost all outer planet spacecraft will be equipped with RTG power sources which require continuous cooling because of their considerable thermal output. Lack of provision for active cooling of the RTGs in either the OPF, VAB, or Mobile Launch Platform (MLP) may preclude installation of the RTGs at the OPF. Other arguments against OPF installation are: cost requirements for continuous and prolonged environmental and safety monitoring following installation. As such, on-pad installation is a viable option. The proposed RTGs weight 85 pounds, are 16 inches in diameter and 23 inches long. They are handled in cumbersome ground transport containers measuring 4 × 6 × 6 feet and weighting 4000 pounds. The external temperature during ground transport (nonvented) approximates 465°F. For these reasons, installation on the pad cannot be accomplished via the orbiter cabin with the payload doors closed.

Although the RTGs may be installed within the Payload Changeout Room (PCR), the duration of the operation, the numbers of personnel required and the limited area available for the installation, indicate that serious particulate and microbial contamination could accrue on the S/C, necessitating recleaning and reassay. The impact this late in the operations would be significant.

3. <u>Pad Failure Changeout Options (Type 2).</u>   This impact could potentially affect mission contingency planning specifically in the time considerations for changeout of the S/C if it fails on the launch pad.   Since this is considered part of the prelaunch ground operation strategy, it should be noted that on-pad changeout has been required for Mariner Mars '69 and '71 and the Mars Viking '75 spacecraft.   In this example, "best turnaround time estimates" are based on removal of just the spacecraft.   The Orbiter and Interim Upper Stage (IUS) remain on the pad.   The estimates are hands-on working times, based on the STS timelines and the 8-hour shift, 2 shift/day, 5 day/week assumptions.   Best case example is based on removal of the S/C and replacement with a launch ready backup and this can be completed in 42 hours.   It is assumed that launch ready means PQ requirements have been met.   However, for a single vehicle mission, removal, repair and reinstallation of the failed S/C is estimated at 72 hours plus repair time.   In this instance, PQ reverification of the S/C (assuming just assay) would increase the estimate by 100% — without considering the repair time or the potential need to reclean the S/C.

4. <u>Launch Complement Assignment (Type 2).</u>   Program planning in the area of launch complement assignment has indicated that the minimum time between launches varied with the number of Orbiters, MLPs and launch pads assigned to the mission.   Six combinations of the three major launch components were considered using the following assumptions: dual launch mission, first launch has been successful, and P/L installation on the pad.   Under these conditions, the best estimates for getting off the second launch ranged from 24 hours (using the full 2-2-2 complement; 2 Orbiters, 2 MLPs, 2 launch pads) to a maximum of 240 hours using a minimal 1-1-1 assignment.   These estimates will be important in determining the proper assignments to meet launch windows.   High assignments (2-2-2) will probably be avoided if possible, since such an assignment would tie up a significant percentage of the space centers launch capability — until a greater number of Orbiters and MLPs are available in the late 80's.

Failure to consider a possible on-pad PQ turnaround requirement could extend the maximum estimate by 30% and the best-time estimate by 300%. Besides the overall impact on planning, specific short-window missions could be jeopardized.

5.   On-Orbit Dwell Time (Type 3).   The available launch period can be described as that set of calendar days during which the injection energy requirement is less than the available launch vehicle injection capability.

Consideration is being given to the option of launching a planetary payload before the launch period opens and waiting in orbit until the optimum injection time.   This will necessitate dwelling in orbit (RTG heat rejection requirement would probably necessitate that the P/L bay doors be open) for extended durations.   The contamination potential for the S/C is increased proportionately; with sources from the exterior of the orbiter, from the operation of the orbital maneuvering and reaction control systems, from previous orbital contaminants, and from within the P/L bay itself.

6.   Spacecraft Deployment (Type 3).   Present planning calls for the Orbiter payload bay doors to be opened within 10-15 minutes after launch.   The S/C can be "committed to deployment" 100 minutes later.   Full consideration has not been given to the potential for recontamination of the S/C based on early deployment.   For example, molecular clouds, off-and-out gassing and sluffing and spaulling from various orbiter elements and several other sources may result in heavy particulate contamination of the S/C.

An additional but separate consideration is the requirement for the remote manipulator to remove RTG cooling jackets prior to deployment. Operationally, the maneuver is hazardous (potential shroud puncture) and the contamination associated with such a removal or the deployment operation itself, is still known.

7.   On-Orbit Repair Option (Type 3).   At present, this concept is not well developed, although it is being considered.   The operational concepts as well as the type and level of contamination associated with any black box repair or replacement on orbit would need to be defined.   The potential PQ impact will be determined when/if this concept matures.

8.   Abort-Relaunch Time Strategy (Type 3).   If an on-orbit abort occurs (orbiter, IUS, or S/C failure) during a single-launch mission or the second launch of a dual-launch mission, it will be necessary to accomplish as rapid a turnaround as possible to meet the launch window.   Best time

estimates, of course, assume that the failure was not S/C related. These estimates do not consider the probable necessity to reclean and reassay the S/C for PQ reasons. Since particles up to 50 micron are ingested in volume during reentry, the planning estimates should be extended accordingly.

9. <u>Launch and On Orbit Profile - Recontamination (Type 3).</u> The STS operations add new dimensions to those experienced during the expendable launch vehicle era. Acoustical and vibrational effects are greater, and on-orbit operations are extended and in the presence of a large launch vehicle which produces contaminants on a continual basis. Several new operations are required, such as deployment. In short, the STS Provides new operations and environments, the effect of which are unknown in the PQ equation.

10. <u>Summary.</u> These problems will be further defined, along with those newly identified, as more information becomes available. As an example, understanding the true impact of on-pad installation of the RTGs requires the knowledge of:

1) the in-use PCR environment,

2) the air changeout capacity of the PCR,

3) the effectivity of the PCR-STS inflatible seal,

4) the turbulent patterns caused by the 2400 square feet of platform, and the GSE,

5) the contaminating effect of these operations within the vertical confines of the P/L bay, and

6) the affect the radiation hazard may have on the duration micro-biological monitoring personnel may spend in the area.

5.1.2.2 <u>STS Proposed Payload Environments.</u> The second activity consisted of reviewing the payload environments proposed by the STS Program Office and identifying potential problem areas, that is, areas where particular or microbial contamination might be excessive, assuming a program where limiting these types of contaminants might be desirable.

The efforts are summarized below:

1. <u>Spacecraft, IUS (Interim Upper Stage) Assembly, and Checkout Operations.</u> Facilities and operations similar to Mariner and Viking missions. Class 100,000 facilities are not optimal but appear adequate. If off-line, PQ considerations can be covered; on-line, no time allotments have been made.

2. <u>Orbiter Prep and Checkout.</u> The Class 100,000 Facilities appear adequate. Cleaning of P/L bay is purported to be in a Class 5000 enclosure, however, minimal time is available; the cleaning requirement is to a visibly clean level but no particle count verification is required. Cleaning occurs early in the process and no time is allotted later in the flow although many operations occur downstream. A P/L bay liner is to be installed to isolate the bay from the superstructure and other systems. The nature of the liner, the capability for cleaning the material, storage, and change cycles are unknown. The liner has 50 micron filters which breathe both ways. Manufacturer and filter media reliability are unknown as is responsibility for leak testing.

Purge capability is provided at 3 sites in the P/L bay; purge gas is HEPA filtered at Class 1000, which is more than adequate. Flow patterns and velocities at payload are unknown. High probability of turbulent flow exists, but these patterns are also unknown. As mentioned, no purge capability exists during hoist in VAB.

3. <u>Transfer of Spacecraft to Pad.</u> Transfer cannister is provided with HEPA filtered, Class 100 purge gas. Transfer occurs on the pad into the PCR which is purported to be a Class 5000 facility. The operational flow pattern is unclean; however, transfer is based on extension of inflatable seals to the cannister from the PCR and a blowdown of the interstitial space. Both of these operations pose problems.

The PCR contains over 2400 square feet of platform area. It also swings away in such a way that it interfaces directly with the outside environment. Cleaning times are totally inadequate and no cleanliness verification tests are presently required. Ground support equipment (GSE) is extensive and several cranes will be required. The number of personnel, the types of

operations and the size of the structure indicate that the PCR poses numerous problems in terms of potential microbial and particulate contamination.

4. <u>Launch and On Orbit Operations</u>. Launch presents new acoustic and vibration environments to the payload. Additionally, the payload is now launched within the payload bay which will be several levels dirtier than the previously cleaned shrouds. Extended orbital operations, especially with P/L bay doors open, pose strong contamination possibilities from numerous sources. Contamination requirements for the orbital phase have been offered by the Contamination Requirements Definition Group. These appear adequate but it is unlikely that the STS can achieve them. Data will not be forthcoming until the first orbital tests in 1979.

5. <u>Reentry</u>. Ingestion of ram air filtered only to 50 micron or larger level. Contamination level totally unacceptable.

6. <u>Summary</u>. In summary, it is concluded that several of the STS environments and operations present potential contaminating events that could jeopardize a spacecraft in which minimization or elimination of microbial burden was a requirement.

5.1.3   Future Activities

Efforts will continue in the area of reviewing Shuttle Program Office plans and alternatives to determine if additional areas of potential impact exist. The evaluation of the applicability of present state-of-the-art microbiological monitoring and verification techniques will be completed. Also, a reassessment of the affect of a protective shroud on identified contaminating events will be completed.

5.1.4   Presentations

Christensen, M.R., "Planetary Quarantine Considerations for Shuttle Launched Spacecraft," presented at the Planetary Quarantine Review, JPL, March 1976.

SECTION VI

PLANETARY BACK CONTAMINATION
(NASA No. 193-58-64)

Contents	Title and Related Personnel
Subtask A para. 6.1	QUARANTINE CONSIDERATIONS FOR SAMPLE RETURN MISSIONS Cognizance: D. Taylor, M. Wardle Associate Personnel: A. Hoffman, J. Jacobs, M. Christensen, J. Bagby (consulta
Subtask B para. 6.2	REMOTE HANDLING TECHNOLOGY FOR EXTRATERRESTRIAL HAZARDOUS MATERIALS Cognizance: R. Koukol, M. Wardle
Subtask C para. 6.3	EXOTHERMIC COATINGS FOR SURFACE STERILIZATION Cognizance: A. Irons Associate Personnel: H. March V. Menichelli

6.1 QUARANTINE CONSIDERATIONS FOR SAMPLE RETURN MISSIONS

6.1.1 Subtask A Introduction

The objective of this task is to identify the quarantine aspects of sample return missions and describe them in terms of mission events and technology needs. While, for purposes of this effort, it is assumed that the outbound segment of such missions are to be handled from a quarantine standpoint in a manner similar to probe or lander missions, the inbound or return portions make them Class 4 missions (Quarantine Provisions for Unmanned Extraterrestrial Missions, NHB 8020.12A, NASA, February, 1976)and present quarantine problems heretofore not resolved.

6.1.2 Significant Accomplishments

Since this task is a new start during FY-76, efforts were directed towards the development of an approach to demarcate quarantine in the context of sample return missions. In order to more effectively define the aspects of quarantine, a particular mission, namely a Mars Surface Sample Return (MSSR) mission was chosen for analysis. A baseline MSSR mission was reviewed to identify the impact of quarantine. The baseline considered was a follows: (1) a single launch from a shuttle craft in 1984, (2) direct Mars entry of the lander, (3) a Mars orbital rendezvous between the Earth return vehicle and the Mars ascent vehicle and (4) direct Earth entry of the return vehicle.

The analysis of the baseline mission led to the identification of nine major quarantine functions related to a MSSR mission. These functions are shown as Figure 6-A.1. It is obvious that each function requires management, and in some cases, technology in its exercise; and that characterization of a function's interdigitation with mission events is of prime importance. Therefore, further understanding of these functions dictates a complete delineation of them from a management and technology needs standpoint, and integration of the functions (including any interaction between or among them) with mission events. Finally, the relationship between quarantine (functions) and various mission options must be considered.

The function entitled "Establish Criteria for Sterilization Decision" has been examined relative to management aspects and technology needs.

- STIMULATE ESTABLISHMENT OF POLICY (U.S. and WORLD)

- DEVELOP NASA REQUIREMENTS AND IMPLEMENTATION PLAN

- ASSURE MISSION COMPLIANCE WITH POLICY

- IDENTIFY CONTAMINATION SOURCES

- DEVELOP, EVALUATE, CONTAMINATION CONTROL TECHNOLOGY

- ESTABLISH, TEST, VERIFY CONTAINMENT REQUIREMENTS

- ESTABLISH CRITERIA FOR STERILIZATION DECISION

- ESTABLISH, EVALUATE, STERILIZATION REQUIREMENTS

- ESTABLISH CRITERIA FOR SAMPLE HANDLING AND RELEASE

Fig. 6-A.1. Major Quarantine Functions

Figure 6-A.2 shows the questions a manager must pose in the enactment of this function. Figure 6-A.3 indicates some of the possible particulars for each management question. The prevailing thought relative to a MSSR mission is that a nominal mission would return an unsterilized sample; however, management must be prepared for a contingency. In addition, it is conceivable that a sterilization decision may entail other than the sample per se. It is the approach of the present task to analyze each function in detail so that the impact quarantine may have on different mission modes can be readily ascertained.

Figure 6-A.4 shows that the technology needs for criteria evaluation are the ones unique to the "sterilization decision" function; such is most likely the case for the other functions with technology needs. As to the what and where management questions, the technology need input will be identified in the analysis of two additional functions; i.e., "Identification of Contaminating Sources" and "Sterilization Requirement." Hence, it can be seen that functions do not stand alone, but rather interrelate. In order to label mission events as

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┌─────────────────────────┐                    ┌─────────────────────────┐
│  WHY IS STERILIZATION   │◄───────────────────│  WHAT ARE STERILIZATION │
│   TO BE IMPLEMENTED?    │                    │        CRITERIA?        │
└─────────────────────────┘                    └─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│     WHAT IS TO BE       │
│       STERILIZED?       │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  WHERE IS STERILIZATION │
│     BEST EXERCISED?     │
└─────────────────────────┘
```
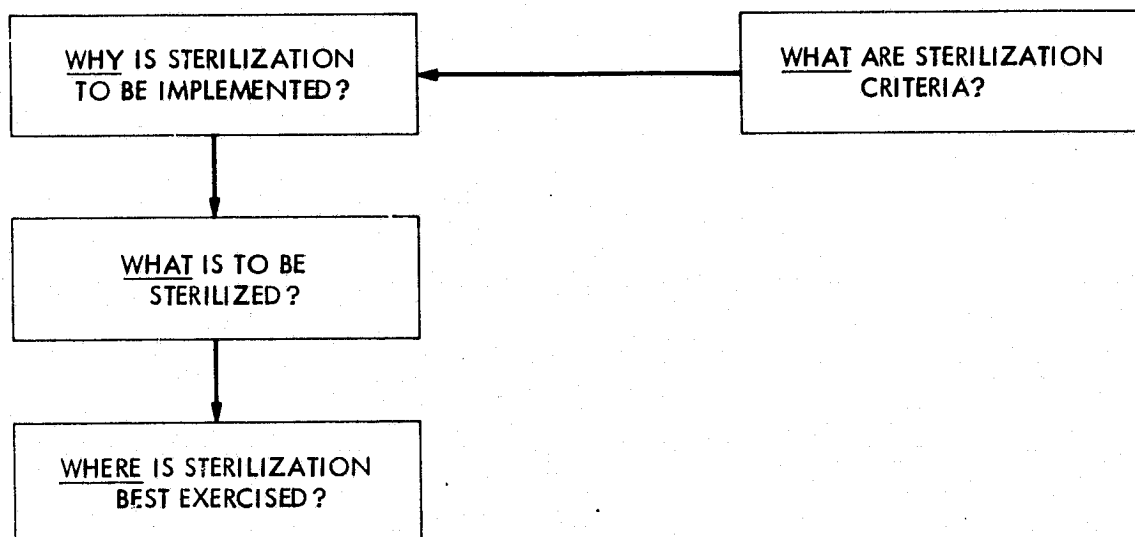
Fig. 6-A.2. Establish Criteria for Sterilization Decision, Management
Questions

having a quarantine significance and therefore a technology need peculiar to
quarantine considerations, the technology needs of each function must be under-
stood relative to one another.

6.1.3   Future Activities

Future work in this task area will involve the completion of analysis
of other sample return quarantine functions and their interrelationships. The
impact of quarantine on mission events and options will be identified in terms
of management and technology needs.

WHY IS STERILIZATION TO BE IMPLEMENTED?	WHAT ARE STERILIZATION CRITERIA?

WHY IS STERILIZATION TO BE IMPLEMENTED?

1.0 SATISFACTION OF 1 OR MORE STER. CRITERIA

2.0 RISK ANALYSIS

WHAT ARE STERILIZATION CRITERIA?

1.0 SAMPLE ACQUISITION

2.0 BIOHAZARD ASSESSMENT

    A. POSITIVE VIABLE LIFE DETECTION (METABOLIC, REPRODUCTIVE)

    B. DETECTION OF SPECIFIC CHEMICAL COMPONENT

    C. ECOSYSTEM(s) CHALLENGE

3.0 SAMPLE IS LOST

4.0 SAMPLE CANNISTER INTEGRITY VIOLATED

5.0 BIOBARRIER OR OTHER CONTAINMENT SYSTEM BREACHED

6.0 POTENTIAL CATASTROPHIC FAILURE OF RETRIEVAL/ TRANSPORT SYSTEM

7.0 RELEASE FROM MRL

WHAT IS TO BE STERILIZED?

1.0 EXTERIOR OF MAV

2.0 EXTERIOR OF ERV

3.0 EXTERIOR OF CANNISTER

4.0 EXTERIOR OF EEC

5.0 INTERIOR OF EEC (TRANSFER CHAMBER)

6.0 SAMPLE

WHERE IS STERILIZATION BEST EXERCISED?

1.0 BASED ON WHERE YOU CAN DEMONSTRATE CRITERIA SATISFIED

2.0 BASED ON CAPABILITY TO EFFECTUATE STERILIZATION AT THAT POINT

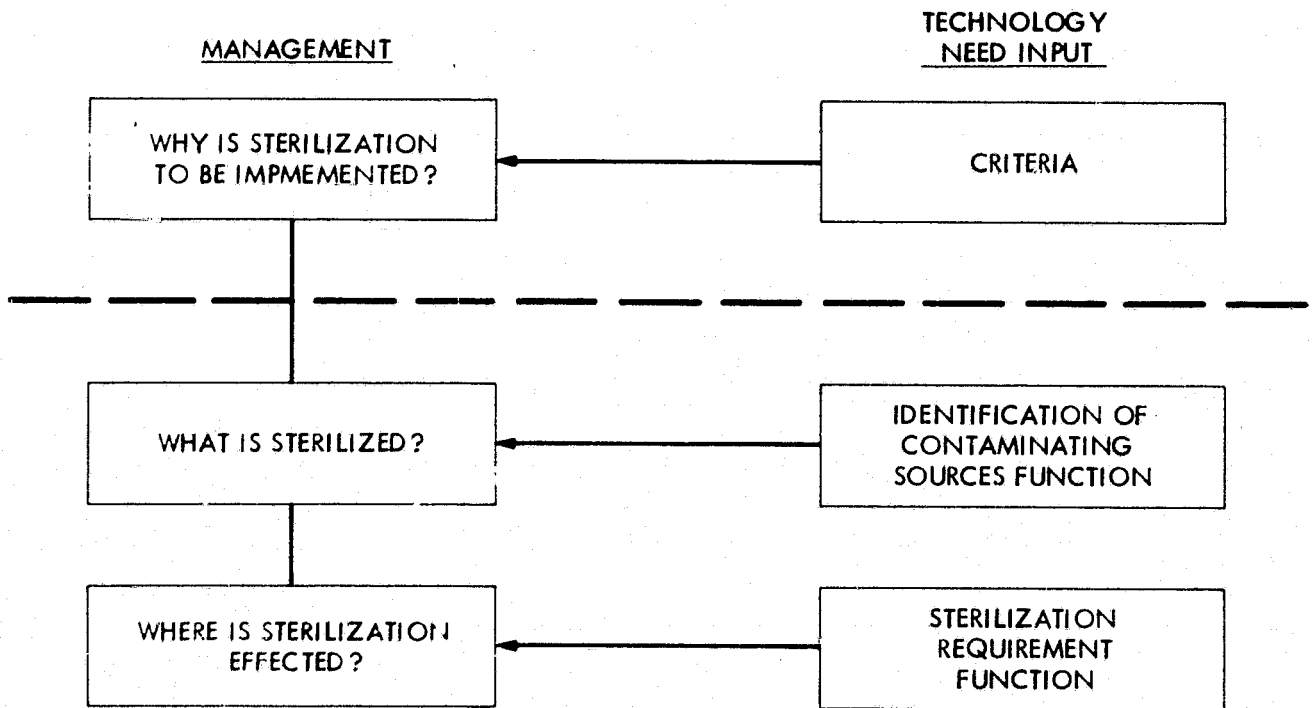Fig. 6-A.3. Establish Criteria for Sterilization Decision, Management Considerations

Fig. 6-A.4. Establish Criteria for Sterilization Decision, Summary

6.2 REMOTE HANDLING TECHNOLOGY FOR EXTRATERRESTRIAL HAZARDOUS MATERIALS

6.2.1 Subtask B Introduction

The objective of this task is to assess the state-of-the-art of remote handling technology in regard to determining its suitability and adaptability for handling extraterrestrial hazardous materials.

A successful Viking mission to Mars may find viable life forms or traces of life on another planet for the first time. A possible follow-on mission would be to return to Mars, obtain a surface sample and return it to Earth for analysis. To preserve the integrity of this sample material for science objectives and to insure that terrestrial quarantine requirements are not compromised, some type of containment system must be used.

A containment system used in the Lunar Receiving Laboratory (LRL) to handle the returned soil samples was found to be deficient in several areas. Since the LRL was considered by some scientists not to be totally satisfactory for lunar samples, the facilities do not appear to be adequate for possibly hazardous biological material that may be represented by a returned sample (Ref. 1). Two of the LRL problems which could be alleviated through the use of remotely manned systems are 1) leakage of gloves utilized in Class III hoods and 2) difficulty in handling small amounts of sample.

6.2.2 Approach

A literature search was performed to determine the types of remote handling systems which are in production, current topics in remote handling research, and theories on future study and development.

Site visits will be made to various facilities involved in the area of remote handling technology. Additional visits will be made to facilities involved in biological research which may be considered analogous to that which will be performed on a sample returned from another planet.

To determine the applicability of the remote handling equipment for manipulating biohazardous material, a set of characterizations has been developed. Thirteen characterizations have been enumerated at this time. From this list a study matrix will be developed which will compare and contrast different remotely manned systems.

6.2.3 Significant Accomplishments

The literature search, which led to several personnel contacts, has been completed. The investigation to date has led to the conclusion that manual or semi-automated remotely manned systems are best suited for biohazardous material handling. Automated systems currently do not have the judgmental ability necessary to handle dangerous material.

A micromanipulator which is designed for handling small amounts of sample material under a microscope has been identified. This system has a positioning sensitivity of less than 0.005 mm.

6.2.4 Future Activities

Site visits will be made to determine the nature of the sample handling process and examine the current status of remote handling technology. After completing the site visits the characterization matrix will be completed for applicable remote handling systems.

6.2.5 Presentations

Wardle, M. D., "Remote Handling Technology for Extraterrestrial Hazardous Materials," presented at the NASA Spacecraft Sterilization Technology Seminar, Cocoa Beach, Florida, August 1975.

6.2.6 References

1. "Technology for Return of Planetary Samples," Biospherics Incorporated, Final Report 1975, Contract No. NASW-2280.

6.3   EXOTHERMIC COATINGS FOR SURFACE STERILIZATION

6.3.1   Subtask C Introduction

There is a possibility of contaminating Earth and the Earth's atmosphere with extraterrestrial life forms which may be carried on the surfaces of space hardware, such as sample return containers, which were in contact with the biosphere of other planets. Life forms carried on surfaces could perhaps be destroyed by the use of exothermic surface coatings capable of producing sterilizing temperatures. These coatings could be activated either spontaneously by the heat generated upon entry into the Earth's atmosphere, or formulated so as to require intentional activation.

6.3.2   Approach

The performance of this subtask consists chiefly in the application of appropriate elements of established pyrotechnic technology to the special requirements of missions in which exterior surfaces of objects returning to Earth's environment from planetary biospheres must be completely free of extraterrestrial organisms before entry. In order to best carry out the objective of the subtask, the following approach has been adopted:

1) Search the literature to identify candidate coatings having the capability of producing surface sterilizing temperatures.

2) Identify precursors required to permit activation and completion of the exothermal reaction.

3) Evaluate methods of application of coatings to ensure complete covering of the area to be sterilized.

4) Investigate coating compatibility with space hardware surfaces.

5) Estimate theoretical surface temperatures generated by various coatings, time to reach temperature, and time at sterilizing temperature.

6) Establish theoretical probabilities of sterility based on the time and sterilizing temperature profiles of the various coatings.

7) An experiment will be designed to permit physical analysis and biological testing of at least one candidate coating material. Methods of application as well as evaluation of substrate composition and design will also be considered.

6.3.3 Significant Accomplishments

A number of pyrotechnic compositions have been identified as potentially suitable for this application. They fall into two classes: gas-forming and non-gas-forming. One special concept, which appears to offer advantages in reliability, utilizes a non-gas-forming composition sandwiched between a thin metal outer covering and the body of the object. The results of a limited literature search are presented.

During and since World War II the Army and Navy have carried out considerable research in the development of pyrotechnic delay compositions. The main purpose of these compositions was to introduce a time delay between a sequence of events, e.g., to obtain safe separation between an explosive load munition and the delivery system, or to obtain penetration of a target before the explosion of a projectile, rocket, bomb or missile. Pyrotechnic delay compositions are also used in self-destruct operations, thermal batteries and for precision delayed switching.

Before applying pyrotechnic compositions to highly sophisticated ordnance devices it was necessary to understand their chemistry and behavior. To this end considerable research was carried out at Picatinny Arsenal, Dover, New Jersey and the Naval Ordnance Laboratory, Silver Spring, Maryland. The Navy also worked three contracts with the Universal Match Corporation, St. Louis, Missouri. A result of this contract led to the development of the United Match Naval Ordnance Laboratory (UMNOL) compositions. This work is reported in Summary Report No. 2, Contract NORD 13466, Task 2, 1958. As a result of this work and more, military specifications for pyrotechnic compositions such as ignition powders (MIL-P-22264), Manganese Delay Composition (MIL-M-21383), Tungsten Delay Composition (MIL-T-23132), etc., are now

available. A typical Manganese Delay Composition consists of manganese powder (10 to 14 microns diameter) barium chromate powder and lead chromate powder. The compositions can be formulated to produce burning times between 2 and 14 seconds per inch. Pyrotechnic compositions possess a series of advantages e.g., they have no moving parts, they are simple to construct, relatively inexpensive, occupy small volumes and are of small mass; they are rugged, reliable, and operate at both low and hight temperatures, i.e., 260-1600°C as well as various pressures.

The technology of delay and igniter compositions developed by the military appears to have direct applicability to surface sterilization. The technology as practiced by the military is to load the compositions into metal tubes in a sequential order so as to obtain reliable and precise delay sequences. The application of these compositions to surface sterilization is ideal. It is well known how to reliably ignite them and the constraints of precision timing are eliminated. The burning rate of the composition will be adjusted for the desired temperature and the total time of reaction will be of secondary consideration.

It is not known at this time what geometry the sample return capsule will take. Let us first consider a simple shape such as a cylinder. In this geometry the pyrotechnic compositions can be dry loaded (ram and press) into a thin metallic shell in physical contact with the capsule or as an integral part of the capsule. If the geometry of the capsule becomes more complex, then pyrotechnic compositions can be combined with a plastic binder such as dialyll phthalate resin and loaded in the manner as a grease gun prior to curing. Another approach could be to apply successive pyrotechnic coats (of pyrotechnic composition, binder, solvent) to the surface until the desired thickness of composition is obtained. In each of the above approaches a rugged, strong composition will result.

Ignition of the composition can be electrical or mechanical. Both are well known and understood. Electrical initiation can be achieved from spacecraft power sources, or the capsule can carry its own energy source e.g., a battery. Mechanical initiation can be achieved by preloading a spring which can be released by some capsule maneuver or by a clock mechanism. Another approach could be to ignite the composition from reentry heating.

There is one major problem concerning the suggested systems, i.e., there is no past experience to refer back to for some of the answers to obvious questions. As a result, experimental investigations must be carried out. For example, if the capsule is externally coated what will be the durability of the coating-to-substrate bond? What will be the adhesiveness of the composition to the capsule material? What will be the affects of long-time space storage on the sensitivity and behavior of these compositions? Questions of this type can only be answered by experimentation.

The intent of the return capsule mission is to determine if some type of life exists and if so to study that life. Therefore, while the external surface of the capsule must be made sterile, the contents of the capsule must be preserved. Knowing the properties of the materials which will make up the capsule will permit determination of the type and quantity of insulation required to isolate the internal contents from the external surface temperature.

6.3.4   Future Activities

Time delay powder compositions and other candidate materials will be subjected to critical screening for the purpose of narrowing the field down to those most likely to meet all requirements. Special considerations which will be employed in screening and the selection of candidate concepts and compositions include: the coating loading density needed, coating adhesion, mechanical ruggedness, flame spreading, initiator design, insulation of substrate, safety, reliability, nature of residual surface and fabrication difficulty. Selected candidate materials will be tested to eliminate unsuitable compositions. Initial elements of the testing program which have been tentatively identified include: coating integrity, surface temperature and temperature distribution during burning, aging, back heating of substrate or substructures and the affect of sterilizing temperatures on the stability of substrate and substructures.

6.3.5   Presentations

None.

SECTION VII

PLANETARY QUARANTINE LABORATORY ASSAY ACTIVITIES (AFETR)
(NASA No. 195-58-63-05)

Contents

Title and Related Personnel

PLANETARY QUARANTINE LABORATORY ASSAY ACTIVITIES (AFETR)

Cognizance: J. R. Puleo

Associate
Personnel: G. Oxborrow
N. Fields
S. Bergstrom
L. Maull
S. Rood (Bionetics)

7.1   PLANETARY QUARANTINE LABORATORY ASSAY ACTIVITIES (AFETR)

7.1.1   Introduction

The objective of this task is to determine and document the quantitative and qualitative microbiological profiles of pertinent automated outbound spacecraft which may carry terrestrial organisms to the planets.

This type of work is required by NASA policy and lays a foundation upon which future policy can be formulated.

7.1.2   Significant Accomplishments

Bioassays were completed on two Viking spacecraft. Microbiological assessments were done on Viking Precursor, Viking 1, and Viking 2. Viking 1 consisted of Viking Lander 1 (VLC-1), Orbiter 1 (VO-1), and Shroud B. Viking 2 consisted of VLC-2, VO-2, and Shroud A. Microbiological samples were taken and assayed at pre-determined milestone events by methods described in the Viking '75 Program Microbiological Assay and Monitoring Plan. Each milestone event consisted of approximately 250 swab samples plus 25 negative controls. Samples were taken from the spacecraft and assayed by the responsible organization and the Planetary Quarantine Laboratory (PQL). The PQL was responsible for assaying 50% of all samples taken as described in the Viking '75 Plan.

Table 7-1 shows the number of swab samples taken on the various Viking spacecraft. A total of 6683 samples were taken and assayed. Of these, 624 were negative control samples. All bioassays were done in the three separate laboratory areas as described in para. 6.1.2 of Jet Propulsion Laboratory (JPL) Doc. No. 900-715, October 1975.

Observed levels of microbial contamination on the Viking Orbiters (VO) are presented in Table 7-2. Because the front surfaces of the solar panels were only sampled once, the results obtained were included in all calculations to arrive at the total number of microorganisms per square foot obtained at the various milestone sampling periods. Table 7-3 shows the microbial contamination detected on the VLC at the various milestones. When estimating the levels

Table 7-1. Number of Samples Assayed for Viking Missions

Source	No. of Samples Taken	No. of Controls	Total No. Assayed
Precursor			
VLC-1	990	104	1094
VO-1	325	33	358
Shroud B	250	25	275
Solar Panels	52	5	57
Subtotal	1617	167	1784
Viking I			
VLC-1	1003	103	1106
VO-1	594	60	654
Shroud B	250	25	275
Subtotal	1847	188	2035
Viking 2			
VLC-2	1251	134	1385
VO-2	792	80	874
Shroud A	500	50	550
Solar Panels	52	5	57
Subtotal	2595	269	2864
TOTAL	6059	624	6683

of microbial contamination on the VO and VLC surfaces, the 50% efficiency factor of the swab-rinse technique was not considered in the mathematical computation used to obtain the final quantitative results.

The data from the cooperative swab assay of the Viking Lander and Orbiter were analyzed to determine whether results obtained by the Planetary Quarantine Laboratory personnel were different from those obtained by the JPL-VO and Martin-Marietta Aerospace-VLC personnel. The results are

Table 7-2. Microbial Contamination Detected on the Viking Orbiters

Source	Milestone	No. of Samples	Microorganisms per square foot	
			aerobes[a]	aerobic spores[b]
VO-1 (P)[c]	O-4	185	234	8
VO-1 (P)	O-5	140	80	6
Solar Panels		52	45	5
VO-1	O-3	250	397	4
VO-1	O-4	250	151	2
VO-1	O-5	250	39	4
Solar Panels		52	19	9
VO-2	O-3	250	826	13
VO-2	O-4	250	113	3
VO-2	O-5	250	21	4
VO-2	O-5R[d]	250	47	2

[a] Samples not heat-shocked; aerobic incubation; count includes solar panel (front surface) counts

[b] Samples heat-shocked; aerobic incubation; count includes solar panel (front surface) counts

[c] Precursor

[d] Recycle; samples retaken due to spacecraft problems

shown in Table 7-4. The data show that if the high single counts are excluded, no significant differences are observed.

Bacterial colonies from each milestone assay were randomly selected and picked for identification according to those limits established (Table 7-5). The total number of colonies isolated from each component of the three crafts sampled is shown in Table 7-6. A total of 294, 460 and 540 colonies were isolated from the Precursor, Viking 1 and Viking 2, respectively. These included organisms recovered from the non heat-shocked portion of the sample as well as those organisms which survived heat shocking. 1294 colonies were picked throughout the program for identification; of these, 951 were vegetative organisms and 343 were spore-forming organisms.

Table 7-3.   Microbial Contamination Detected on the Viking Lander
Capsules (VLC) and Shrouds

Source	Milestone	No. of Samples	Microorganisms per square foot	
			aerobes[a]	aerobic spores[b]
VLC-1 (P)[c]	C-3	250	-	22
VLC-1 (P)	C-4	251	-	74
VLC-1 (P)	C-5	293	99	19
VLC-1 (P)	C-6	196	1816	13
Shroud B (P)	C-7	250	63	2
VLC-1	C-3	253	-	14
VLC-1	C-4	250	-	15
VLC-1	C-5	250	21	3
VLC-1	C-6	250	12	5
Shroud B	C-7	250	22	4
VLC-2	C-3	250	-	21
VLC-2	C-4	250	-	9
VLC-2	C-5	250	225	4
VLC-2	C-6	250	22	2
Shroud A	C-7	250	12	5
VLC-2	C-6R[d]	251	17	1
Shroud A	C-7R[d]	250	187	3

[a]Samples not heat-shocked; aerobic incubation
[b]Samples heat-shocked; aerobic incubation
[c]Precursor
[d]Recycle; samples retaken due to spacecraft problems

Each organism was examined microscopically and placed into one of
several large groups. Further testing along with computer analysis of the data
collected, assigned each organism to a more specific group. The percentages
and types or organisms identified from each craft are shown in Tables 7-7,
7-8 and 7-9.

Table 7-4.   Comparison of Quantitative Results Obtained from Viking
Bioassay Conducted by PQL, JPL and MMA

ORBITER

PQL Colony Count		JPL Colony Count	
aerobes[a]	aerobic spores[b]	aerobes[a]	aerobic spores[b]
3236[c]	52	1545	36

LANDER

PQL Colony Count		MMA Colony Count	
aerobes[a]	aerobic spores[b]	aerobes[a]	aerobic spores[b]
1011	357	4291[d]	658[e]

[a] Samples non heat-shocked; aerobic incubation

[b] Samples heat-shocked; aerobic incubation

[c] Total affected by a single high count of 1941 - Subtracting this count from the total yields 1295.

[d] Total affected by a single high count of 3303 - Subtracting this count from the total yields 988.

[e] Total affected by a single high count of 309 - Subtracting this count from the total yields 349.

Members of the genus Bacillus were the most frequently isolated organisms during assay of the Precursor (Table 7-7).   These accounted for slightly over 47% of the total isolates identified and is more than twice the number of any other group isolated.

Over 55% of the organisms detected on the Viking 1 spacecraft were Gram positive cocci, (Staphylococcus spp. and Micrococcus spp.).   These

Table 7-5. Selection of Isolates for Identification

Total No. Colonies per Milestone	Number of Colonies Selected
0-20	100%
21-99	20[a]
≥ 100	20%[b]

[a]From Milestones C4, C6 and VO-5, all colonies not to exceed 100, were selected.

[b]Not to exceed 100 colonies.

Table 7-6. Number of Microorganisms Identified and Lyophilized

Spacecraft			NHS[a]	HS[b]
PRECURSOR	VLC-1			
		Milestone C-3	-	19
		Milestone C-4	-	31
		Milestone C-5	54	16
		Milestone C-6	89	7
			143	73
	SHROUD B			
		Milestone C-7	26	2
	VO-1			
		Milestone O-4	26	4
		Milestone O-5	19	1
			45	5
		Subtotal	214	80

[a]Samples not heat-shocked; aerobic incubation

[b]Samples heat-shocked; aerobic incubation

Table 7-6. Number of Microorganisms Identified and
Lyophilized (contd)

Spacecraft			NHS[a]	HS[b]
VIKING 1	VLC-1			
		Milestone C-3	-	14
		Milestone C-4	-	79
		Milestone C-5	29	6
		Milestone C-6	26	10
			55	109
	SHROUD B			
		Milestone C-7	18	7
	VO-1			
		Milestone O-3	82	7
		Milestone O-4	72	3
		Milestone O-5	86	8
		Solar Panels	12	1
			252	19
		Subtotal	325	135
VIKING 2	VLC-2			
		Milestone C-3	-	19
		Milestone C-4	-	45
		Milestone C-5	45	7
		Milestone C-6	44	5
		Milestone C-6R	26	3
			115	79
	SHROUD A			
		Milestone C-7	22	11
		Milestone C-7R	69	6
			91	17

[a]Samples not heat-shocked; aerobic incubation
[b]Samples heat shocked; aerobic incubation

Table 7-6. Number of Microorganisms Identified and
Lyophilized (contd)

Spacecraft			NHS[a]	HS[b]
VIKING 2 (contd)	VO-2			
		Milestone O-3	56	22
		Milestone O-4	27	2
		Milestone O-5	30	6
		Milestone O-5R	86	0
		Solar Panels	7	2
			206	32
		Subtotal	412	128
		TOTAL	951	343

organisms are characteristic of those being indigenous to human skin, hair, and respiratory tract and are constantly being expelled from the human body. The level of spore-formers (Bacillus spp.) and Actinomycetes, which are associated with soil and dust, was found to be low (Table 7-8) when compared with other automated spacecraft.

The profile of organisms detected on the Viking 2 spacecraft was very similar to that seen on Viking 1 (Table 7-9). Micrococcus spp. and Staphylococcus spp. constituted the greater percentage of microorganisms isolated from VO-2. A previously undetected microorganism, belonging to the genus Streptococcus, and probably of human origin, was isolated from the Orbiter. A greater diversity of species was seen among the Bacillus identified.

The above data did not reflect the members of the genus Bacillus which were isolated and identified from heat-shocked assay samples. Although that data was not reflected in the microbial profiles of each spacecraft sampled (Tables 7-7, 7-8, 7-9), it was collected and compared to the Bacillus obtained from non heat-shocked samples. The only major difference observable is in the frequency of isolation of organisms identified as Bacillus subtilis. Approximately 17% of the Bacillus spp. isolated and identified from non heat-shocked portions of assay samples was classified as B. subtilis. The number

Table 7-7. Percentages[a] and Types of Microorganisms Detected
on the Viking Precursor[b]

Type	Lander	Orbiter	Shroud	Total
Staphylococcus spp.				
Subgroup II	0.7	4.3	0	1.4
Subgroup III	0	0	0	0
Subgroup IV	0	4.3	4.2	1.4
Subgroup V	1.4	0	0	0.9
Subgroup VI	0	0	0	0
Micrococcus spp.				
Subgroup 1	2.1	0	12.5	2.8
Subgroup 2	0	0	4.2	0.5
Subgroup 7	9.0	4.3	0	7.0
Subgroup 8	0	0	0	0
Atypical Micrococcus	6.2	8.7	25.0	8.8
Bacillus spp.				
B. alvei	0	0	0	0
B. brevis	4.1	0	0	2.8
B. cereus	0.7	0	0	0.5
B. circulans	0	4.3	4.2	1.4
B. coagulans	0	0	0	0
B. firmus	1.4	0	4.2	1.4
B. laterosporus	0	0	0	0
B. lentus	8.3	13.0	8.3	9.3
B. licheniformis	0.7	0	0	0.5
B. macerans	0	0	0	0
B. megaterium	1.4	2.2	4.2	1.9
B. polymyxa	0	0	0	0
B. pumilus	5.5	0	4.2	4.2
B. sphaericus	2.1	2.2	0	1.9
B. subtilis	13.8	6.5	12.5	12.1
Atypical Bacillus	11.0	13.0	8.3	11.2
Corynebacterium-Brevibacterium group	20.7	32.6	4.2	21.4
Actinomycetes	4.1	4.3	4.2	4.2
Yeasts	6.9	0	0	4.7
Total No. Isolated	143	45	26	214

[a]Includes only non-heat shocked, aerobic, mesophilic organisms.

[b]Precursor consists of VLC-1, VO-1 and Shroud B.

Table 7-8. Percentages[a] and Types of Microorganisms Detected on the Viking 1[b]

Type	Lander	Orbiter	Shroud	Total
Staphylococcus spp.				
Subgroup II	1.8	10.3	10.0	8.8
Subgroup III	0	2.4	0	1.8
Subgroup IV	7.2	8.3	5.0	7.9
Subgroup V	0	4.7	10.0	4.3
Subgroup VI	0	0.8	0	0.6
Micrococcus spp.				
Subgroup 1	1.8	3.6	0	3.0
Subgroup 2	3.6	0.8	0	1.2
Subgroup 7	18.2	17.4	10.0	17.1
Subgroup 8	5.5	0	0	0.9
Atypical Micrococcus	7.3	10.7	5.0	9.8
Bacillus spp.				
B. alvei	3.6	0.4	5.0	1.2
B. brevis	0	0.8	0	0.6
B. cereus	3.6	0.8	0	1.2
B. circulans	0	0.8	5.0	0.9
B. coagulans	0	0	0	0
B. firmus	1.8	1.6	0	1.5
B. laterosporus	0	0	0	0
B. lentus	9.1	5.5	10.0	6.4
B. licheniformis	0	0	0	0
B. macerans	0	0	0	0
B. megaterium	0	1.6	0	1.2
B. polymyxa	0	0	0	0
B. pumilus	3.6	0.4	0	0.9
B. sphaericus	0	0	0	0
B. subtilis	5.5	1.2	10.0	2.4
Atypical Bacillus	5.5	7.1	20.0	7.6
Corynebacterium-Brevibacterium group	16.4	17.4	10.0	16.8
Actinomycetes	1.8	0.8	0	0.9
Yeasts	3.6	2.8	0	2.7
Total No. Isolated	55	252	18	325

[a]Includes only non-heat shocked, aerobic mesophilic organisms

[b]Viking 1 consists of VLC-1, VO-1 and Shroud B.

Table 7-9. Percentages[a] and Types of Microorganisms Detected
on the Viking 2[b]

Type	Lander	Orbiter	Shroud	Total
Staphylococcus spp.				
Subgroup II	8.1	2.9	2.2	4.2
Subgroup III	0.8	0.5	0	0.5
Subgroup IV	3.3	22.9	9.9	14.4
Subgroup V	2.4	6.7	19.8	8.3
Subgroup VI	0	1.0	0	0.5
Micrococcus spp.				
Subgroup 1	1.6	1.9	8.8	3.3
Subgroup 2	0	0.5	3.3	0.9
Subgroup 7	26.8	10.0	4.4	13.7
Subgroup 8	0	0	1.1	0.2
Atypical Micrococcus	8.1	22.9	7.7	15.3
Streptococcus-Enterococcus group	0	0.5	0	0.2
Bacillus spp.				
B. alvei	0.8	0	0	0.2
B. brevis	0.8	1.4	0	0.9
B. cereus	1.6	1.0	1.1	1.2
B. circulans	1.6	0.5	0	0.7
B. coagulans	0.8	0	0	0.2
B. firmus	0.8	0.5	1.1	0.7
B. laterosporus	0	0	0	0
B. lentus	4.9	3.8	8.8	5.2
B. licheniformis	0.8	0	0	0.2
B. macerans	0.8	0	0	0.2
B. megaterium	0	1.0	0	0.5
B. polymyxa	0	0.5	0	0.2
B. pumilus	0.8	0.5	3.3	1.2
B. sphaericus	0	0.5	0	0.2
B. subtilis	4.1	1.4	6.6	3.3
Atypical Bacillus	7.3	4.3	17.6	8.0
Corynebacterium-Brevibacterium group	16.3	13.3	3.3	12.0
Actinomycetes	7.3	1.4	0	2.8
Yeasts	0	0.5	1.1	0.5
Total No. Isolated	115	206	91	412

[a]Includes only non-heat shocked, aerobic, mesophilic organisms.

[b]Viking 2 consists of VLC-2, VO-2 and Shroud A.

of organisms identified as B. subtilis from heat-shocked portions of the samples dropped noticeably and accounted for only 8% of the Bacillus spp. identified (Figure 7-1). Over 50% of the organisms recovered, whether from heat-shocked or non heat-shocked portions of assay samples, were distributed into two major groups, namely, Bacillus lentus and the atypical Bacillus spp. This distribution is not uncommon and has been observed in previous assays of spacecraft.

A comparison of the percentage and types of microorganisms isolated from the Viking spacecraft is shown in Table 7-10. Approximately 75% of isolates detected on Viking 1 and 2 spacecraft were those considered to be indigenous to humans (Micrococcus spp., Staphylococcus spp. and Corynebacterium-Brevibacterium group). The remaining microorganisms were associated with soil and dust in the environment. The Precursor, however, was found to have a greater percentage of those microorganisms associated with soil and dust.

After identification, the microbial isolates were lyophilized and stored for future reference. Cultures were grown on Trypticase Soy Agar slants, harvested and suspended in sterile double strength skim milk. A 0.5 ml aliquot of the bacterial suspension was aseptically transferred into steril 2 ml glass vials for lyophilization. The lyophilization system employed was a Virtis Freeze Dry Unit (Model 10-145MR-BA) and Virtis Model 10-MR-SA drying chamber. After lyophilization, the vials were flame sealed under 10-15μ vacuum. The integrity of the vacuum was confirmed by use of a vacuum leak detector (Electri-technic Products, Model No. 8D10).

The 1294 cultures were lyophilized in triplicate and arranged in numerical order according to their cellular morphology. The vials were placed into boxes and stored in a freezer at -20°C. The 3882 vials will be stored in the PQL and, periodically, randomly selected cultures will be examined for viability.

7.1.3   Future Activities

Future work will be directed to bioassay of pertinent automated outbound spacecraft (i.e. Mariner Jupiter/Saturn) as directed by the Planetary
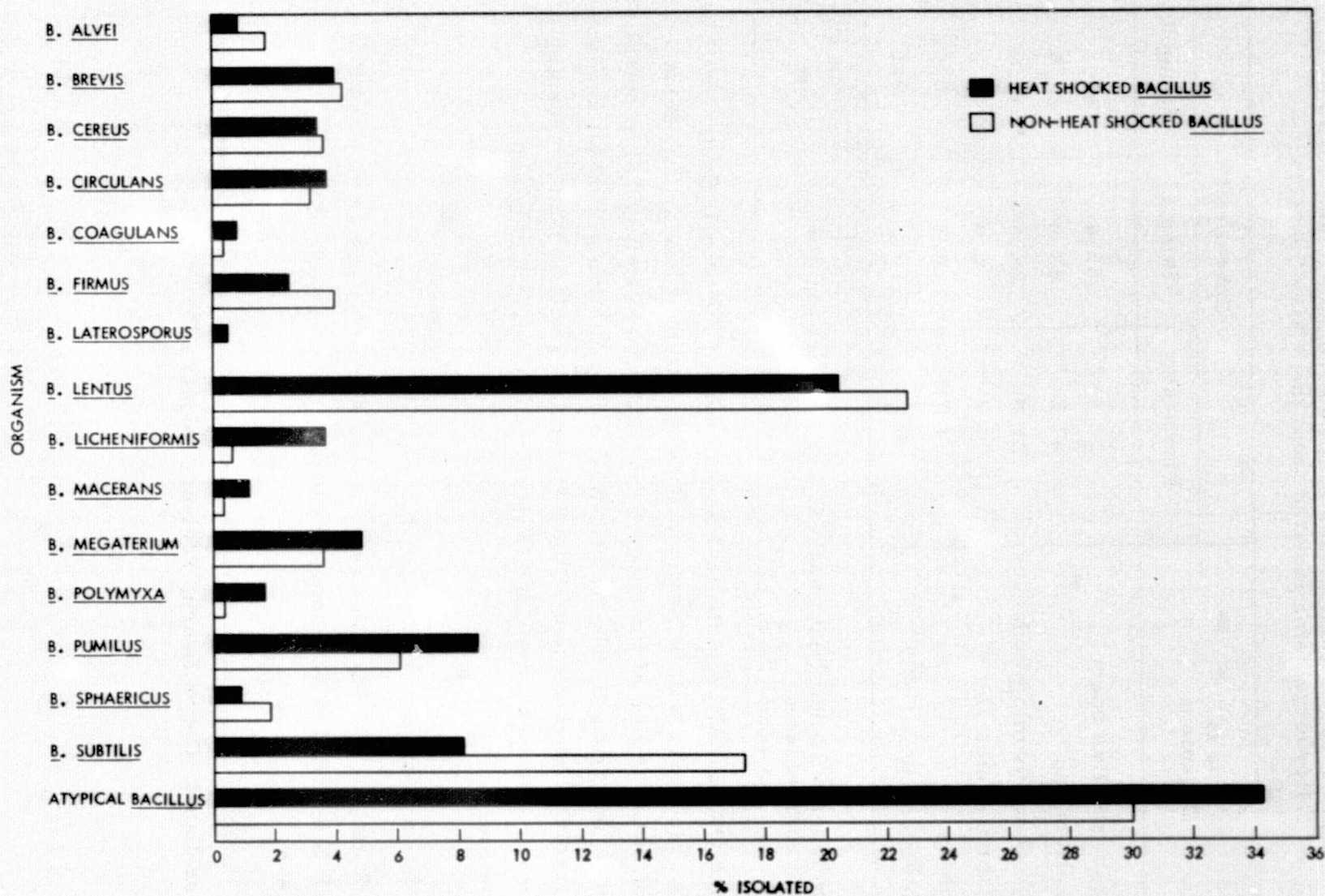
Fig. 7-1. Comparison of Heat Shocked and Non-Heat Shocked Bacillus Isolates Recovered from Viking Spacecraft

900-732

Table 7-10. A Comparison of the Percentages[*] and Types of
Microorganisms Isolated from the Viking Spacecraft

Type of Organism	Spacecraft		
	Precursor	Viking 1	Viking 2
Gram positive cocci	22.9	55.4	61.3
Bacillus spp.	47.2	23.9	22.9
Non-Sporeforming Rods	21.4	16.8	12.0
Actinomycetes	4.2	0.9	2.8
Yeasts	4.7	2.7	0.5
Enterococcus	0	0	0.2

[*]Includes only non-heat shocked, aerobic, mesophilic
microorganisms

Quarantine Office. Microbiological assessment of the Shuttle System will be
initiated. Bioassays will be performed on the Payload Bay and Spacelab
Module of initial flights of the Space Transportation System (STS) to establish
a biological background. The quantitative and qualitative microbial assessment
of the intramural environment of the STS facilities will be done. Cleaning
procedures will be evaluated for efficiency in removing biological
contamination.

7.1.4   Presentations

Puleo, J. R., PQ Laboratory Assay Activities (AFETR), NASA
Spacecraft Sterilization Technology Seminar, Cocoa Beach, Florida,
August 1975.

Puleo, J. R., Status of Viking Isolate Storage, AIBS Planetary
Quarantine Panel, Cocoa Beach, Florida, August 1975.

SECTION VIII

PLANETARY QUARANTINE LABORATORY - RESEARCH
ACTIVITIES
(NASA No. 193-58-63-06)

Title and Related Personnel

TEFLON RIBBON EXPERIMENTS

Cognizance: J.R. Puleo

Associate
Personnel: G. Oxborrow
                N. Fields
                S. Bergstrom

PYROLYSIS GAS-LIQUID
CHROMATOGRAPHY STUDY

Cognizance: G. Oxborrow

Associate
Personnel: N. Fields
                J.R. Puleo

8.1   TEFLON RIBBON EXPERIMENTS

8.1.1   Subtask A Introduction

The objective of this study is to characterize the thermal resistance profiles of naturally occurring bacterial spores associated with assembly facilities at Kennedy Space Center.

The validity of the currently accepted sterilization cycle should be confirmed. The cycle, to be valid, should be effective on bacterial spores associated with spacecraft in residence at Kennedy Space Center, Florida.

8.1.2   Significant Accomplishments

The teflon ribbon study was discontinued in May due to the Viking '75 Program. It will resume in January '76.

Due to modification of the high bay area in the Vehicle Assembly Building (VAB), the teflon ribbons were removed from the 20th floor of B tower, and relocated on the 27th floor of D tower on 30 December, 1975.

A meeting was held in the Planetary Quarantine Laboratory on Dec. 1-2 to review the status of the teflon ribbon study and to determine what additional work is needed to complete the study. Attendees were Dr. J. E. Campbell, FDA, Mr. T. Peeler, FDA, Mr. P.S. Stabekis, Exotech System, Inc. and members of the PQL. The consensus of the group was that additional information was needed in the following areas: a) effect of relative humidity, b) effect of temperature, and c) effect of time at temperature. The study will be completed by September '76.

8.1.3   Future Activities

Future work will be directed to:

1)   characterize the thermal resistance profiles of naturally occurring bacterial spores from assembly facilities at KSC.

2)   develop thermal death curves using various temperatures, water concentrations and duration of thermal exposure.

3)   develop and field evaluate assay techniques and procedures.

4)   continue cooperative studies with Hardin-Simmons University to determine the occurrence of mesophilic and psychrophilic anaerobic microorganisms with unusual thermal resistance.

5)   continue cooperative studies with the Food and Drug Administration to determine thermal inactivation of homogenous and heterogenous bacterial spore populations.

6)   characterize the thermal resistant actinomycetes recovered from the teflon ribbon study, and determine their heat inactivation kinetics.

7)   determine thermal inactivation kinetics of bacterial spore populations isolated from the Viking Lander Capsule and Orbiter spacecraft.

8.1.4   Presentations

1.   Puleo, J. R., Gordon S. Oxborrow and Norman D. Fields, "Evaluation of a Terminal Sterilization Process," presented at the Southeastern Branch of the American Society for Microbiology, Tampa, Florida, November 21 and 22, 1975.

2.   Bergstrom, S. L. and John R. Puleo, "Identification of Heat-Stressed Environmental Isolates," presented at the Southeastern Branch of the American Society for Microbiology, Tampa, Florida, November 21 and 22, 1975.

8.1.5   Publications

Puleo, J. R., M. S. Favero, G. S. Oxborrow and C. M. Herring, "Method for Collecting Naturally Occurring Airborne Bacterial Spores for Determining Their Thermal Resistance," Applied Microbiology, Vol. 30, No. 5, November 1975.

8.2 PYROLYSIS GAS-LIQUID CHROMATOGRAPHY STUDIES

8.2.1 Introduction

The objective of this investigation is to assess the usefulness of pyrolysis gas-liquid chromatography (PGLC) as a rapid reliable method of characterization or identification of members of the genus Bacillus.

The variability in the morphological and biochemical data obtained from the members of the genus Bacillus, particularly those encountered in the terminal decontamination studies, makes identification inconclusive. Forty to seventy percent of the isolates were identified as atypical after conventional tests had been applied. PGLC has been shown to give consistent identification of Salmonella, Mycobacterium and several other genera.

8.2.2 Significant Accomplishments

Preliminary results reported in para. 7.2.2 of Jet Propulsion Laboratory (JPL) Doc. No. 900-715, October 1975, were verified using a 1/8 inch × 20 foot glass column packed with 10% carbowax 20m TPA on Anakrom ABS 100-110 mesh support. All cultures were grown using the membrane filter technique described in para. 7.2.2.1 of JPL Doc. 900-715.

Pyrochromatograms obtained from six Bacillus species grown on media prepared from six lot numbers of Trypticase Soy Agar (TSA) were compared. Little or no difference was observed on the chromatograms obtained from cultures grown on the different lot numbers of TSA. Figure 8-B.1 shows the results obtained for B. cereus grown on the six lot numbers of TSA media. Similar results were obtained with the other five Bacillus species. Approximately 74 peaks were resolved in these chromatograms as compared to 43-47 previously obtained on the stainless steel column.

Six Bacillus spp. grown on different types of culture media, namely TSA, Eugon Agar, and Nutrient Agar, were compared to assess the chromatographic differences. The results confirmed the preliminary data which showed several differences, particularly in peak areas most significant for species identification. Differences encountered in the chromatograms of B. firmus and B. subtilis var. niger are shown in Figure 8-B.2. Peaks 3, 6, 7, 11 and 16
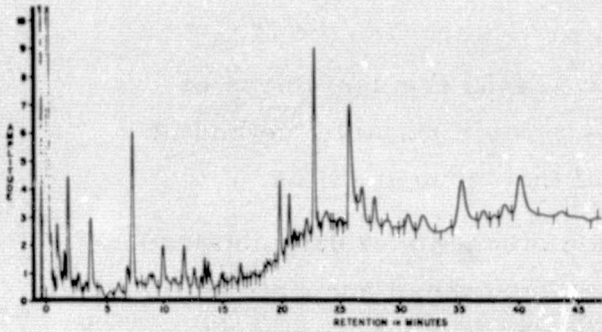
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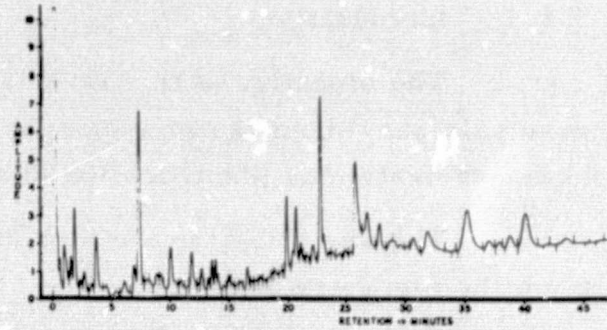
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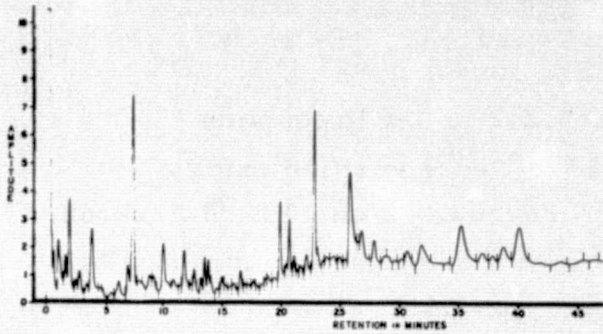
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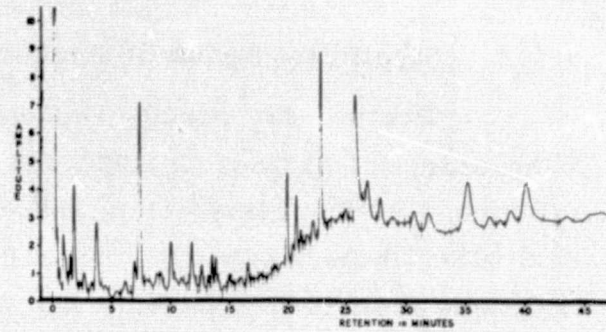
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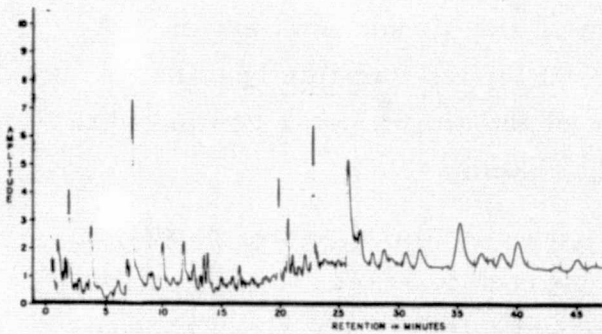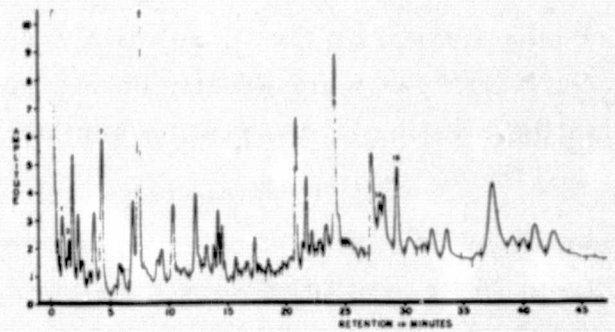
B. cereus 6472, TSA lot# L6DCSF
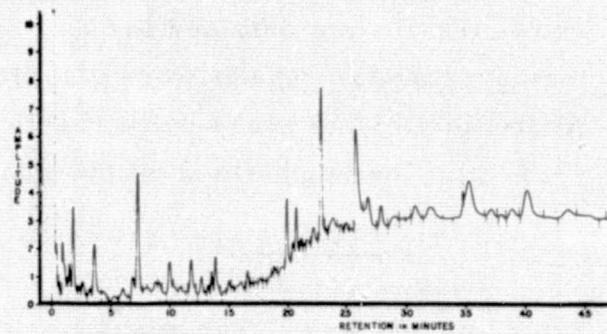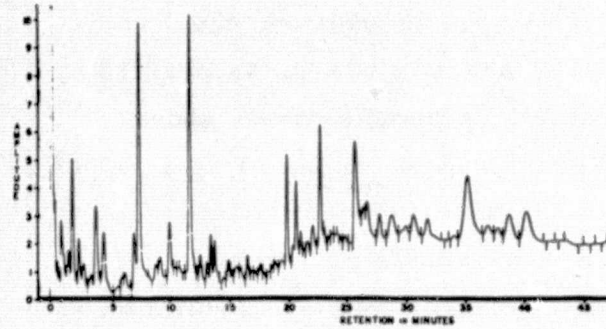
B. cereus 6472, TSA lot# K6DCMI

Fig. 8-B.1. Comparison of Pyrochromatograms of B. cereus 6472 Grown on Six Lot Numbers of Trypticase Soy Agar (TSA)
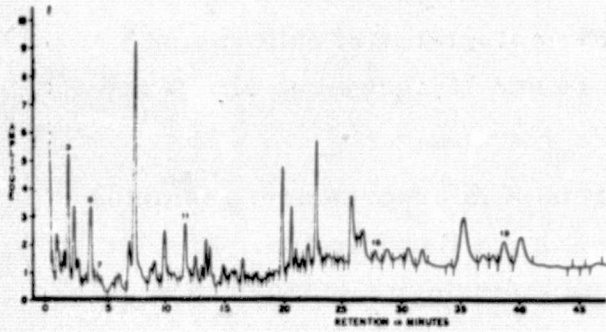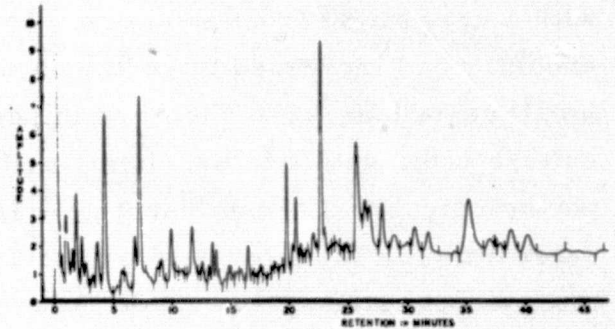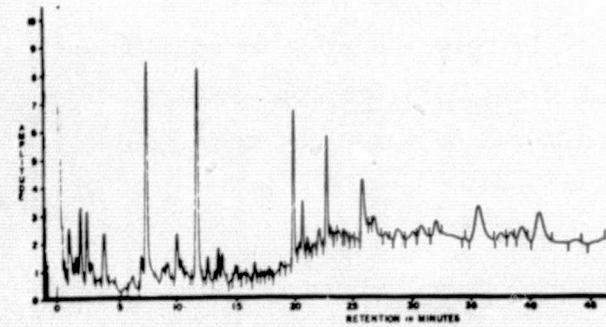
B. firmus 749, Trypticase soy Agar

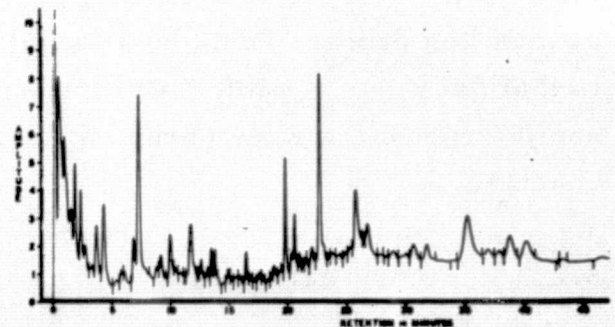B. subtilis   Trypticase soy Agar
var. niger,

B. firmus 749, Eugon Agar

B. subtilis   Eugon Agar
var. niger,

B. firmus 749, Nutrient Agar

B. subtilis   Nutrient Agar
var. niger,

Fig. 8-B.2. Comparison of Pyrochromatograms of B. firmus 749 and
B. subtilis var. niger Grown on Three Different Media

on the chromatograms of B. firmus were not consistent for the media tested. Peaks 1 and 2 on the B. subtilis var. niger chromatograms of cultures on Nutrient Agar were greatly enhanced. Peaks 14 and 16 showed no similarity in their ratios of one peak to another.

A study to compare pyrochromatograms of microorganisms sampled in the various growth phases was completed using six Bacillus spp. Results from 24 hr. cultures were consistent as long as culturing techniques were constant. When cultures were allowed to age, or proceed from one growth phase to another, the chromatograms changed. Figure 8-B.3 shows the results obtained for B. cereus. The ratios of peak 12 to 13 and 15 changed most between 96 hrs. and 7 days. Peak 18 began at 24 hrs. as a single peak with a very slight front shoulder. At the end of 96 hrs. incubation, the shoulder had increased to become a distinct peak, nearly as large as peak 18. Small variations were observed in peaks 19 and 20. A progressively older culture of B. subtilis var. niger is shown in Figure 8-B.4. Peak 11 is a physiological marker in that it gets larger with the onset of sporulation. To determine the occurrence of bacterial spores at the various growth periods, microscopic stains were made for each chromatographic sample. Examination of these slides did not reveal the presence of any bacterial spores after 23 hrs. of growth. At 36 hrs., less than 0.1% spores were observed. The sporulation process became more rapid at 39 hrs. and 40 hrs. when approximately 1-2% spores were seen. By the 66th hr., 50-60% of the cells had sporulated and many of the cells were beginning to fragment. The early detection of the sporulation process could be a useful tool in physiological studies or identification of the genus Bacillus. Chromatograms of the six Bacillus spp. studied were easily reproduced when chromatographic and cultural parameters were held constant.

8.2.3   Future Activities

Future activities will be directed to: 1) continue pyrolysis of known bacterial cultures and environmental microbial isolates to complete a chromatographic catalogue, 2) determine correlation between physiological characteristics and peak variation, 3) identify or characterize dry-heat resistant microorganisms, 4) assess the effect of culturing techniques, 5) evaluate a
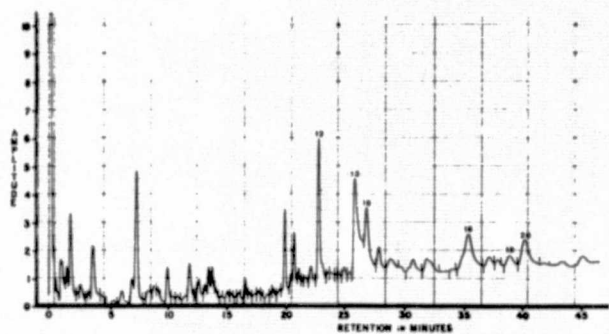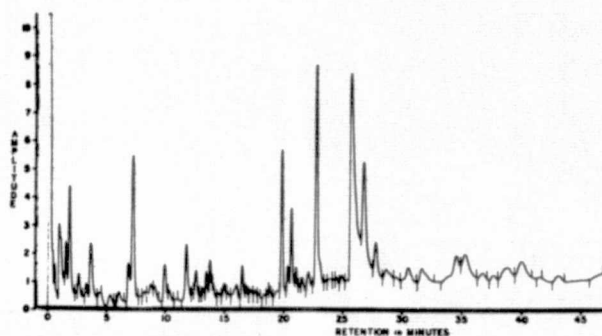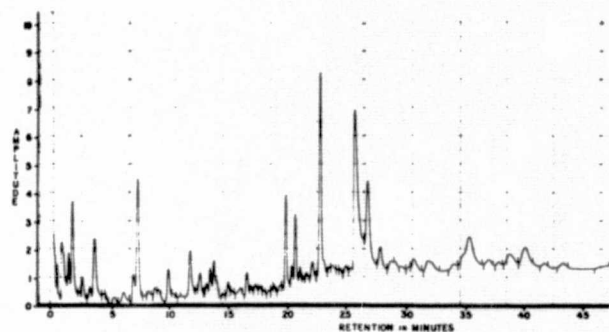
B. cereus 6472, 24 hr. @ 32C
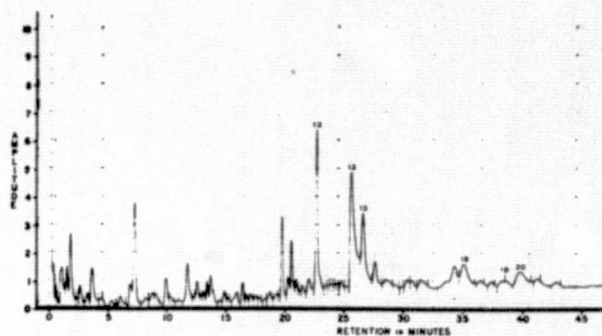
B. cereus 6472, 96 hr. @ 32C
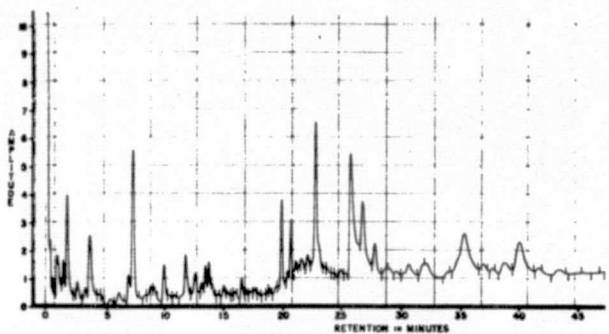
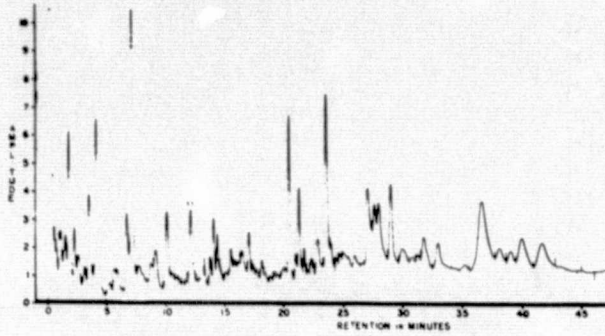B. cereus 6472, 48 hr. @ 32C

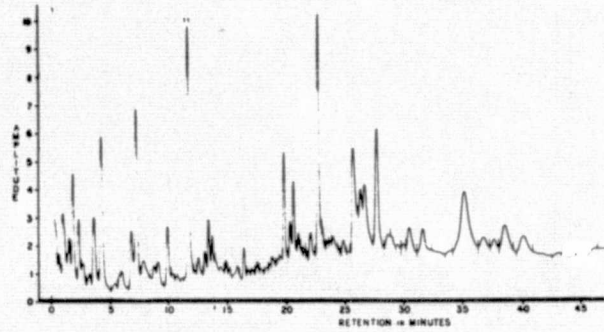B. cereus 6472, 7 days @ 32C

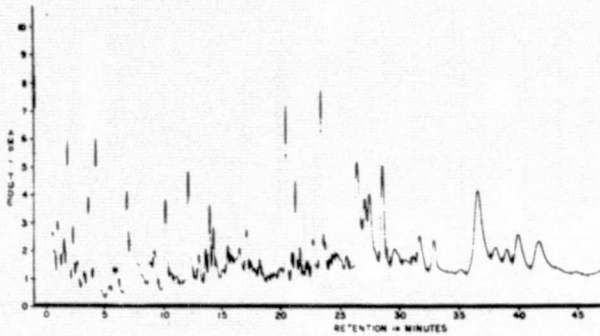B. cereus 6472, 72 hr. @ 32C

Fig. 8-B.3.   Effects of Culture Age on Pyrochromatograms
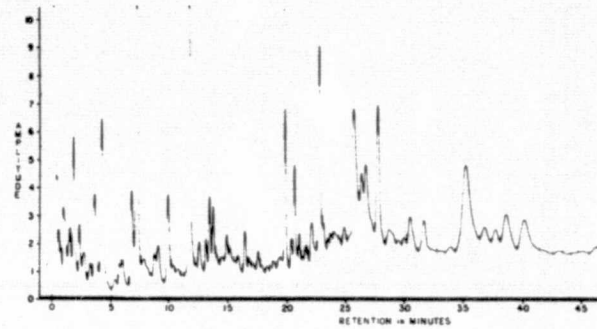of B. cereus 6472

B. subtilis   23 hr. culture
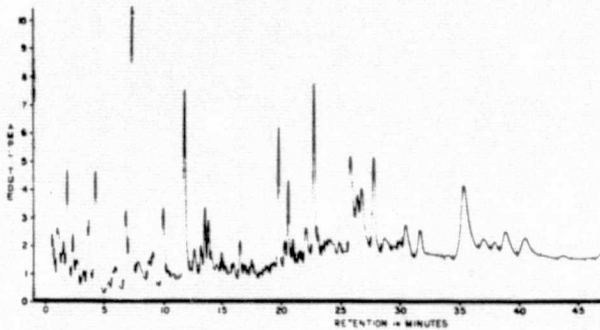var. niger,

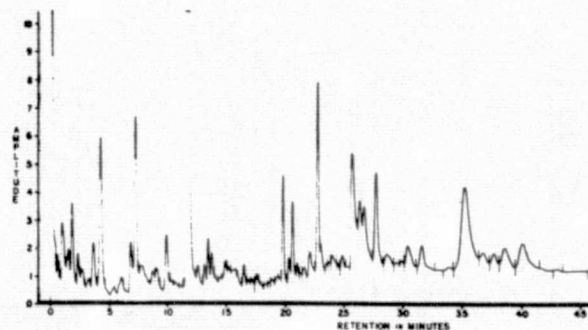B. subtilis   40 hr. culture
var. niger,

B. subtilis   36 hr. culture
var. niger,

B. subtilis   42 hr. culture
var. niger,

B. subtilis   39 hr. culture
var. niger,

B. subtilis   66 hr. culture
var. niger,

Fig. 8-B.4.   Effects of Sporulation on Pyrochromatograms
of B. subtilis var. niger

pattern recognition computer identification system, 6) chemically identify resultant chromatographic peaks using mass spectroscopy, and 7) initiate a cooperative studies with Hardin-Simmons University for characterizing mesophilic and psychrophilic anaerobic microorganisms.

8.2.4   Presentations

Oxborrow, G.S., N.D. Fields, and J.R. Puleo, "Procedures for Pyrolysis Gas-Liquid Chromatography of Microorganisms," presented at the Southeastern Branch of the American Society of Microbiology, Tampa, Florida, November 21 and 22, 1975.