Paper No. 16

THE EFFECT OF 1 TO 5 keV ELECTRONS ON THE REPRODUCTIVE INTEGRITY OF MICROORGANISMS

J. Barengoltz, *Jet Propulsion Laboratory, California Institute of Technology, and* J. Brady, *The Bionetics Corporation*

ABSTRACT

Microorganisms were exposed to a simulated space environment in order to assess the effect of electrons in the energy range 1 to 5 keV on their colony-forming ability. The test system consisted of an electron gun and power supply, a dosimetry subsystem, and a vacuum subsystem. The system was capable of current densities ranging from 0.1 nA/cm^2 to 5 μA/cm^2 on a 25 cm^2 target and an ultimate vacuum of 6 x 10^{-4} N/m^2 (4 x 10^{-6} torr). The results of the experimental program have shown a significant reduction in microbial reproductive integrity.

INTRODUCTION

Planetary quarantine is an international cooperative program concerned with the prevention of the contamination of another planet or satellite of a planet by terrestrial organisms. Such a contamination by an automated spacecraft could yield false positive results from its own life detection instruments and, if the terrestrial microbes grew and spread, would confuse all subsequent studies (Reference 1).

As a part of this program, research is conducted to determine the lethal effect of various natural space environments on microorganisms. In particular the study presented in this paper is directed towards determining the spacecraft-associated microbial burden reduction inherent in a mission history attributable to solar wind electrons.

An electron source system has been constructed to provide exposures to electrons in the energy range 1 to 5 keV in a vacuum. The energy range was selected on the basis of a minimum energy for biological effectiveness (Reference 2) and measurements and models of the energy spectrum (References 3 and 4). Parametric tests in this energy range have been conducted on typical spacecraft microbes at accelerated dose rates (flux) to permit realistic mission doses (fluence) in acceptable test durations. At each energy and dose rate tests with varying doses have been performed and survival curves obtained.

Experimental Apparatus

The Solar Wind Electron Source (SWES) system consists of three subsystems: an electron gun subsystem, a dosimetry subsystem, and a vacuum subsystem (Figures 1 and 2).

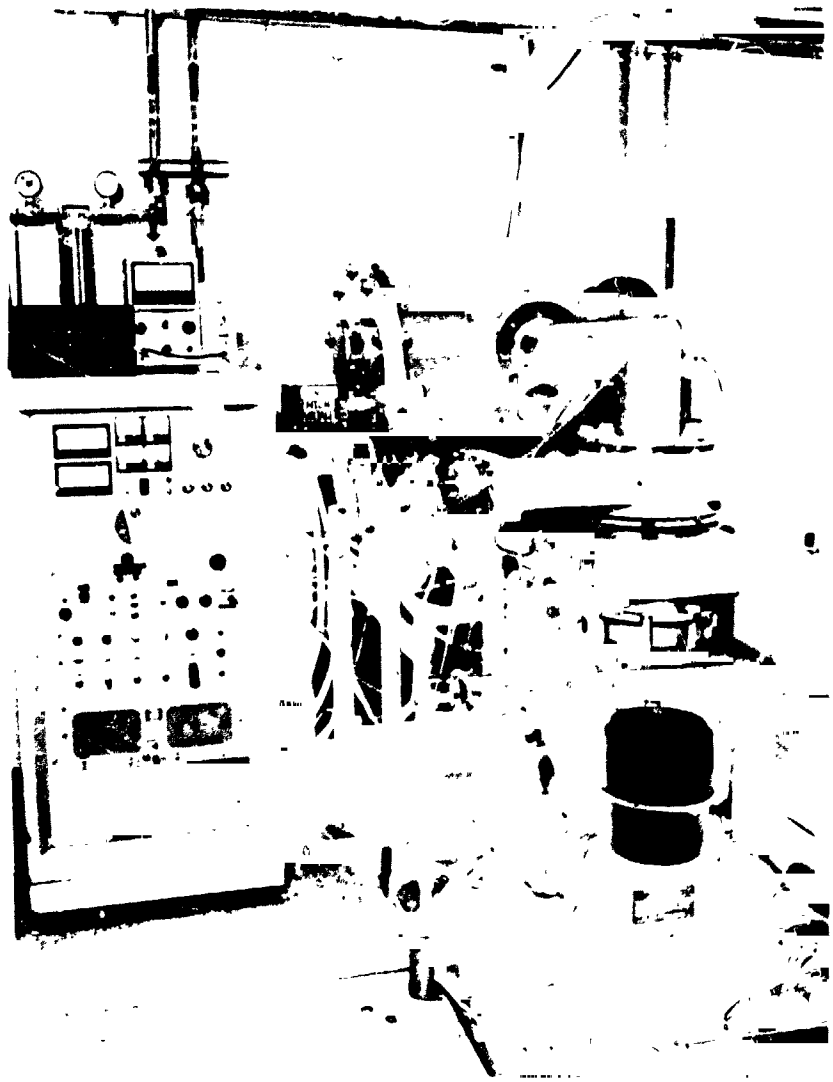

Figure 1. Solar wind electron source, overview of system

The electron gun and its power supply and instrumentation package comprise the electron gun subsystem. The electron gun, visible in the upper right hand corner of Figure 2, may be mounted on the vacuum chamber centerline (as shown) or at one of four off-axis positions. The two-axis gimbal mount permits the beam to be centered in either case. This capability will allow combined environment experiments to be conducted in the future.

The electrons are initially emitted at thermal energies by the "uni-potential high temperature" cathode held at a large negative potential

C-3

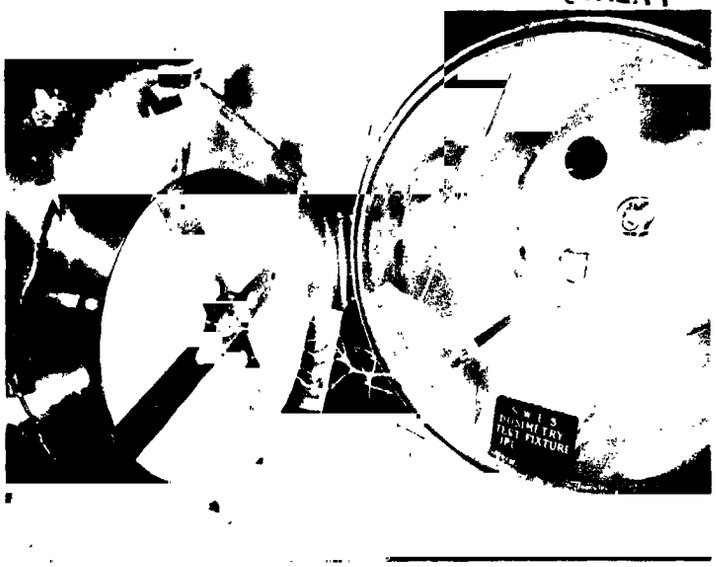

Figure 2. Solar wind electron source, interior view showing dosimetry fixture and electron gun

(the accelerating voltage or energy) with respect to the chamber ground. The cathode emission level is adjustable to change the dose rate. The spatial profile of the beam (divergence) is varied by a solid angle electrode. A pre-acceleration element accelerates the electrons to an intermediate energy before they gain the desired total energy in falling to the test fixture (ground). The power supply and instrumentation package (Figure 1) allows adjustment and monitoring of all of the previous parameters, as well as a beam current monitor.

The electron gun subsystem can produce electrons in the energy range of 1 to 5 keV (±5 percent) at current densities variable from 0.1 nA/cm^2 to 5 μA/cm^2. This current density range is equivalent to a flux (dose rate) range of 6 x 10^8 to 3 x 10^{13} e/cm^{2}s. With the present configuration, the subsystem can cover a circular area of 25 cm with a 20 percent overall radial uniformity and a local uniformity (azimuthal) of 10 percent. A straightforward modification of the electron gun by the addition of a pre-acceleration grid and a ground grid provides a much larger radially uniform coverage at the cost of larger inhomogeneities.

The dosimetry subsystem consists of a dosimetry test fixture, a monitor Faraday cup, a Keithley Model 610B electrometer, and the necessary cabling, vacuum feedthroughs, and connectors. The dosimetry test fixture is comprised of an array of four Faraday cups mountable on 10, 20, or 30 cm diameter circles on a phosphor screen plate. These Faraday cups, together with the monitor Faraday cup on the beam axis, provide a direct reading of the incident current density (proportional to flux) at discrete locations. The cup currents are monitored by the electrometer. The phosphor screen glows under electron bombardment to provide a check of local uniformity and overall profile of the beam. It

may be inspected through a vacuum window at the gun end of the chamber (Figures 2 and 3). The use of the dosimetry subsystem is described below.

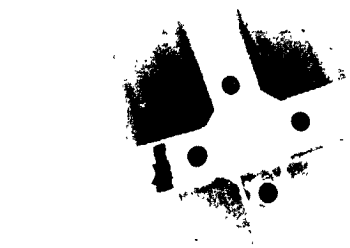

Figure 3. View of dosimetry test fixture through vacuum window with the source in operation

The vacuum subsystem is a 61 cm inner diameter x 70 cm long cylindrical vacuum chamber pumped by a liquid nitrogen trapped 6 in. diffusion pump stand (Figure 1). The subsystem is capable of obtaining a vacuum of 1×10^{-3} N/m^2 (8×10^{-6} torr) from ambient pressure (dry nitrogen) in 1 hour or less. The oil backstreaming, of particular importance to cathode life and beam uniformity, was measured by quartz crystal microbalance techniques over 63 hours of segmented vacuum operation with approximately two hours of gun operation. The deposition rate either exposed to the beam or near the pump inlet was less than 5×10^{-11} g/cm^2 s.

Experimental Procedures

1. Beam Profile and Dosimetry Measurements. The measurements of the beam profile and other dosimetry matters were accomplished with the use of the dosimetry subsystem described above. The dosimetry test fixture was mounted inside the vacuum chamber access door and the cup cables connected (see Figure 2). A complete profile at a particular energy and flux was obtained by consecutive runs with the movable Faraday cups placed on the 10, 20, and 30 cm diameter circles. These runs were normalized for comparative purposes by resetting all controls by the meters and then adjusting the cathode emission precisely with the monitor (center-fixed) Faraday cup.

For the pure culture tests to be discussed, only a single beam mapping run with the movable cups on the 10 cm diameter circle was required. This dosimetry run, which was performed before each experimental run, allowed an optimization of azimuthal symmetry on the circle corresponding to the cultured organism test fixture annulus. When the proper control settings had been noted, the monitor Faraday cup reading to produce the desired flux at the sample annulus was also recorded. During an experimental run, with the dosimetry test fixture replaced by the cultured organism test fixture (Figure 4), the flux was monitored by the monitor Faraday cup. Note that the test fixture has a hole on the beam axis to allow the cup to see the beam. The required fluence was then obtained by timing the duration of the exposure.

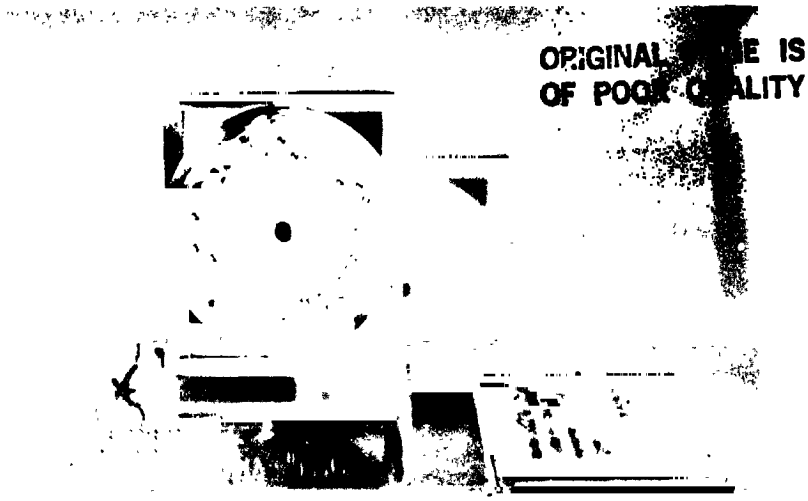

Figure 4. Cultured organism test fixture

2. Microbiology. Pure cultures of MM71 Proof Test Module (PTM) isolates, Bacillus subtilus var. niger (BSN) and Staphylococcus epidermidis (SE) (ATCC-17917) were prepared. Sporeforming organisms were sporulated in the liquid synthetic medium of Lazzarini and Santangelo (Reference 5) modified by the addition of 25 mg of both L-methionine and L-tryptophan to one liter of medium. Mature spores were harvested and washed (7 times with sterile distilled water) by centrifugation (10 min at 9750 relative centrifugal force) with final suspension in distilled water. Nonsporeformers were maintained on Trypticase Soy Agar (TSA; BBL) slants. Lawns were prepared by resuspension of the slant growth in Trypticase Soy Broth (TSB; BBL) and inoculation of TSA plates with the suspension. Plates were incubated at 37°C for 48 hours, and cells harvested (on the day they were to be irradiated) by washing the plates with 20 ml of sterile distilled water. The resulting suspension was washed

with sterile distilled water 4 times by centrifugation (10 min at 9750 relative centrifugal force).

Six sterile aluminum stages were inoculated by micropipette with an amount of each suspension to place about 10^6 microbes on each stage. These stages were allowed to dry in a controlled environment and then mounted on both sides of the test fixture (Figure 4).

After the test exposure, the fixture was assayed in a rapid, consistent manner. Stages were removed from the fixture, placed individually into tubes containing 10 ml of 0.1 percent sterile peptone water, and exposed to ultrasonic treatment (25 kHz for 12 min) in an ultrasonic bath. Upon removal from the bath the tube contents were thoroughly mixed prior to 10-fold serial 1.0 ml dilutions and triplicate 1.0 ml platings of designated dilutions with TSA. The organisms were incubated at 37°C (nonsporeformers) or at 32°C (spores) for 48 hours under dark conditions.

Dilution plates with 30 to 300 colony-forming units (CFU) were enumerated for survivors after the incubation period. Survival fractions were computed by ratioing the bacterial population recovered from the exposed stages to that from the "dark" side stages (controls). These populations were expressed as geometric means. Each experimental run was repeated 4 times. The final survival fractions are arithmetic averages over the 4 replicate runs.

RESULTS AND DISCUSSION

The results of the formal test program are presented graphically in Figures 5 through 12. 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 nonsporeformer (MEAN VEG) survival fractions (Figures 6, 8, 10, and 12).

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 and about 0.1 in the case of mean nonsporeformers at all energies.

The plateau effect is tentatively explained by sample clumping and the resultant shielding of individual organisms. It is well known that electrons in 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 (References 6 and 7). 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 nonsporeformers, dominated by the results for No. 5.

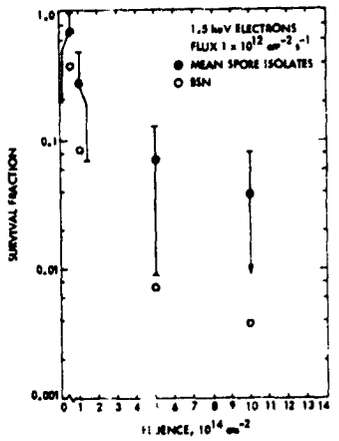

Figure 5. Spore survival at 1.5 keV electron energy

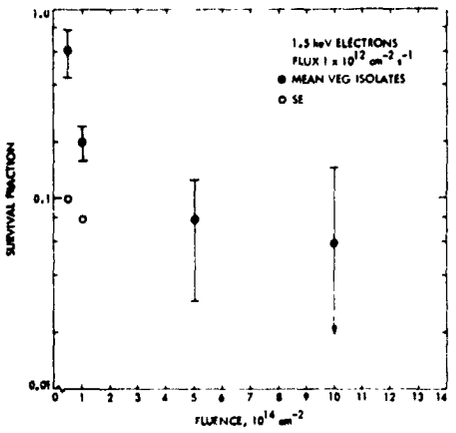

Figure 6. Nonsporeformer survival at 1.5 keV electron energy

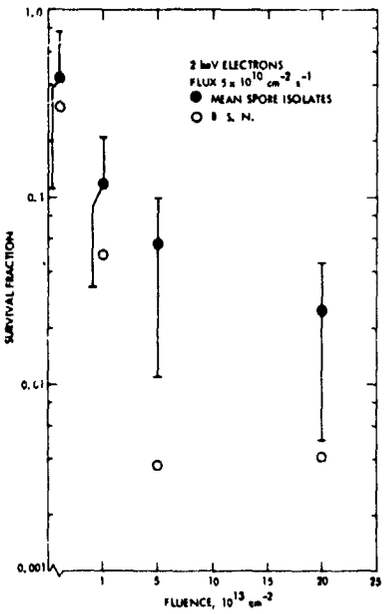

Figure 7. Spore survival at 2.0 keV electron energy

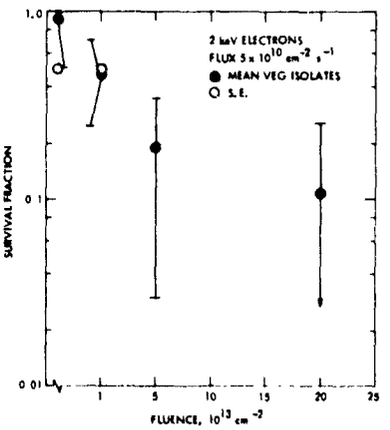

Figure 8. Nonsporeformer survival at 2.0 keV electron energy

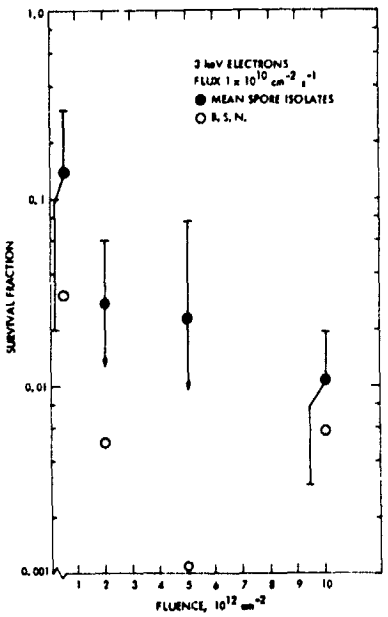

Figure 9. Spore survival at 3.0 keV electron energy

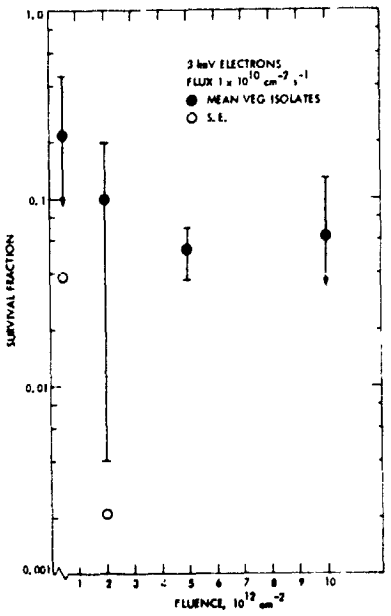

Figure 10. Nonsporeformer survival at 3.0 keV electron energy

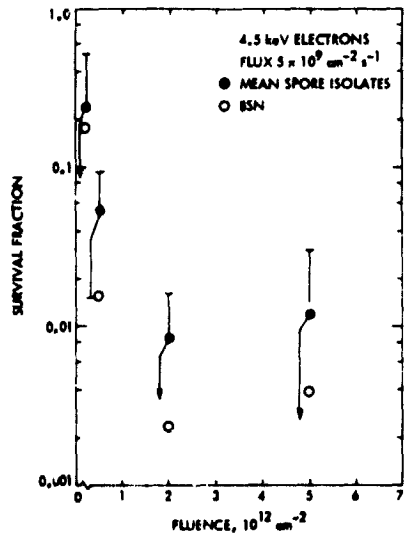

Figure 11. Spore survival at 4.5 keV electron energy

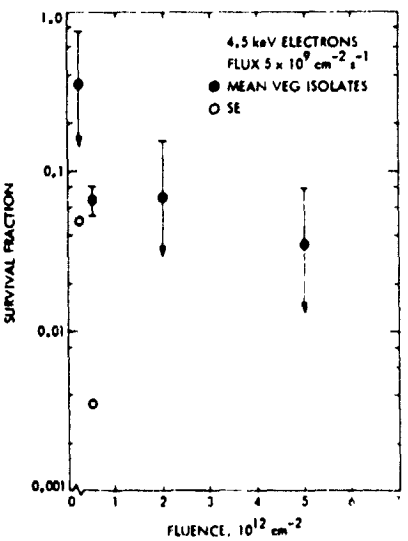

Figure 12. Nonsporeformer survival at 4.5 keV electron energy

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 in the survival fraction. Calculated values from the linear regression analysis for four fluence values are given in Figure 13. 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 B. subtilis as measured by Davis (Reference 2) 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.

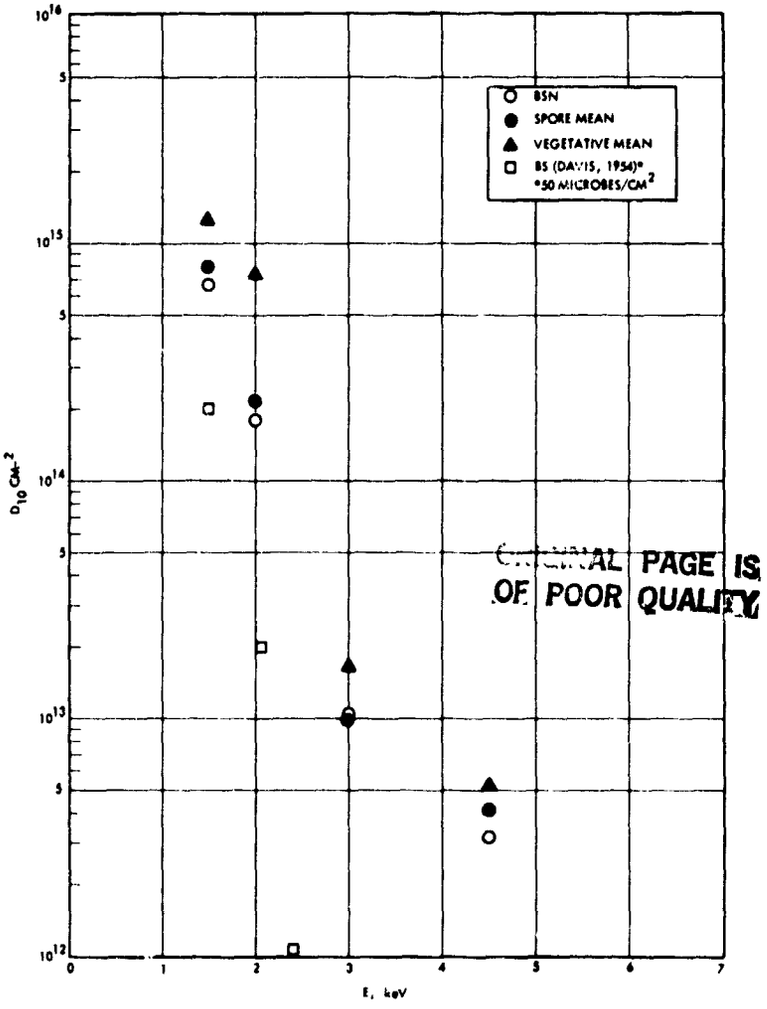

Figure 13. D_{10} values (fluence) as a function of electron energy

ACKNOWLEDGEMENTS

The technical support of A. Ferreira in the construction and operation of the electron source system is deeply appreciated. The authors also wish to thank S. Donovan, J. Farber, G. Simko and S. Yamada for performing the microbiology. The design and fabrication of the electron gun and power supply and the dosimetry fixture by Dr. C. Crawford of Kimball Physics, Inc. is gratefully acknowledged.

This work presents the results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract no. NAS 7-100, sponsored by The National Aeronautics and Space Administration.

REFERENCES

1. Hall, L. B. and Lyle, R. G., "Foundations of Planetary Quarantine," Planetary Quarantine Principles, Methods, and Problems, ed. L. B. Hall, Gordon and Breach (1971), p. 5.

2. Davis, M., Arch. Biochem. and Biophys. 48 (1954), p. 469.

3. Divine, T. N., "Interplanetary Charged Particle Environments," NASA TM 33-637 (1973).

4. Montgomery, M. D., Bame, S. J., and Hundhausen, A. J., J. Geophys. Res., Space Physics 73, No. 15 (1968), p. 4999.

5. Lazzarini, R. A. and Santangelo, E., J. Bacteriology 94 (1967), p. 125.

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

7. Davis, M., Physical Review 94 (1954), p. 243.