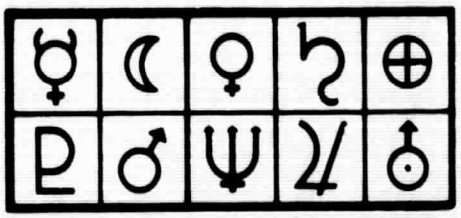

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THE FEASIBILITY OF THERMORADIATION FOR STERILIZATION OF SPACECRAFT - A PRELIMINARY REPORT

Marcel C. Reynolds
Planetary Quarantine Applied
Science Division 1742

SANDIA LABORATORIES

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ABSTRACT

This report presents preliminary data on the feasibility of using combinations of heat and radiation for sterilization of spacecraft. Initial tests indicate that, utilizing relatively low levels of heat and radiation, the technique provides effective bacterial sterilization. The applicability of the technique to a selection of other resistant bacteria, the optimization of temperature/radiation exposures, and the effectiveness on actual spacecraft hardware will be examined in the future.

ACKNOWLEDGMENT

The author wishes to express appreciation to K. F. Lindell, D. M. Garst, J. P. Brannen, and R. H. Staat for assistance in completing this work.

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THE FEASIBILITY OF THERMORADIATION FOR STERILIZATION OF SPACECRAFT - A PRELIMINARY REPORT

Introduction

The employment of dry heat to effect sterilization of spacecraft has resulted in a serious problem in thermal degradation of spacecraft hardware. This problem has been partially resolved through considerable effort on the part of NASA, its contractors and suppliers. Stringent qualifications* for components have been established and a Preferred Parts List of Reliable Electronic Components has been published. In spite of this effort, certain heat-labile components will not withstand present sterilization cycles. Examples of these are[2]: silver-zinc batteries, high-value mylar capacitors, vidicon tubes, tantalum capacitors (10 to 15 μf), solid propellants (especially PBAA), culture media, photometers, and other scientific instruments.

In a study of the effects of dry heat sterilization on reliability, Bartholomew and Porter[3] found the reliability of germanium devices to be the most questionable. Only a very few of these were ever rated at temperatures in excess of 100°C. This is significant in cases where it is desirable or necessary to replace the more heat tolerant silicon devices with the more efficient and radiation resistant germanium devices. Dry heat sterilization of capacitors was approached with skepticism based on the belief that all dielectrics wear out by subtle chemical change that is accelerated by heat or voltage. In addition, many capacitors employ liquids or liquid impregnants that evaporate as a function of time and temperature. Although many capacitors such as the glass, mica, vitreous enamel, teflon, film, air, and vacuum types, were likely to be unaffected by sterilization, all of these have rather poor

*Six 92-hour periods of nonoperational storage at 135°C in an inert atmosphere without degradation.[1]

capacitance-per-unit-volume efficiency factors. The types with rather high volume efficiency factors, such as tantalum, tantalum foil, and ceramic, were of greater concern. Leakage current in the solid electrolyte tantalum capacitors did not seem to be affected by sterilization temperatures of 110°C for periods as long as 300 hours, but did begin to show degradation at 135°C for 36 hours. The dissipation factor showed a similar trend. Tests of the tantalum foil capacitors showed some increase in dissipation factor at 110°C. The nickel cadmium batteries were successfully sterilized at 135°C. This battery, however, has only half of the watt-hour capacity per pound of the more efficient but very heat sensitive silver-zinc cell.

An alternate method of sterilization is the use of ionizing radiation. But radiation sterilization presents a problem similar to that found with thermal sterilization: the radiation exposure levels required—2.5 to 5.0 Mrads (H_2O)—would cause degradation of components. In fact, one might expect as much degradation from radiation exposure as is experienced in the dry heat sterilization cycles at 125° or 135°C.

This study will explore the feasibility of using combinations of heat and radiation where synergistic effects may be available to achieve effective sterilization of spacecraft. This document reports the initial phase of the investigation which showed that the combination of heat and radiation exposure, particularly at low levels, can provide effective bacterial sterilization. In later phases of this study, we plan to examine thermoradiation effects on other organisms, to optimize temperature/radiation exposures, and to investigate potential application of the technique to sterilization of spacecraft hardware.

Historical

A number of investigators have in the past considered the temperature dependency of radiation sterilization of bacteria. Lea, et al., (1936)[4] found little difference in the surviving fraction of Bacillus mesentericus spores exposed to alpha-particles from polonium at temperatures of 50°, 20°, 2°, and -20°C. Similar effects were observed with spores of the same organism when they were exposed to beta-particles from radium at temperatures of 41°, 20°, and -20°C.

Edwards, et al., (1954)[5] irradiated Bacillus subtilis spores with 2 MeV electrons from a Van de Graff accelerator to determine the effects of variation in number of microorganisms, temperature, pH, salt concentration, and the presence of protein. Initial populations considered were 1×10^4 and 1×10^7 spores per ml in buffered saline. Temperature affects were evaluated at 44°, 0°, and -65°C. They found that population, within the ranges considered, had little effect on cell death. The temperature of the suspending medium, however, had a marked influence on the percentages of spores surviving irradiation. Exposure of cells at elevated temperatures resulted in decreased kill while low temperature produced increased destruction. They also found no survivor variation when the radiation dosage was kept constant and the time and intensity of radiation were varied.

Using high-energy cathode rays produced by a 3-MeV Van de Graff accelerator, Proctor, et al. (1955)[6] found that spores of B. subtilis were more sensitive when irradiated at -78°C than at 4.4°C. This was consistent with the work of Edwards, et al. (1954). Proctor also found an increase in the radioresistance of B. subtilis when it was irradiated in the dry state.

Kan, Goldblithe, and Proctor (1957)[7] found that irradiation of Bacillus cereus spores sensitized them to subsequent heat treatment, and Levinson and Hyatt (1960)[8] also found a complementary effect of ionizing energy and thermal energy with Bacillus megaterium.

Webb, et al., (1960)[9] reported that the radiation sensitivity of dry spores of B. megaterium is temperature-independent between -268° and -145°C but is temperature-dependent between -145° and 35°C. Over the latter range the sensitivity increases with increasing temperature during irradiation. During irradiation at temperatures higher than 35°C, radiation sensitivity drops unexpectedly to a minimum at about 80°C.

Graikoski (1961)[10] studied the role of temperature during irradiation on the subsequent survival of B. subtilis, Clostridium botulinum, and Clostridium sporogenes. He found that when anerobic bacterial spores were exposed to gamma radiation in the temperature range of -70° to 95°C, the spores were slightly more resistant to radiation at -70° than at 4°C and reached maximum resistance just below the

thermal lethal threshold for the particular organism under investigation. The thermal lethal threshold for C. botulinum was found to be 85°C. In the case of putrefactive anaerobic spores, the greatest resistance was observed at a temperature of 85°C. Alteration of the radiation dose did not alter thermal lethal thresholds.

Licciardello and Nickerson (1962)[11] found no apparent differences in the thermal resistances of C. sporogenes PA 3679 spores in phosphate buffer when they were irradiated with gamma from a cobalt-60 source over a dose range of 0 to 660,000 rep (555,000 r ds H_2O) and at temperatures between -78° and 20°C. Further, doses of 165,000 and 330,000 rep produced no significant differences between 20° and 66-68°C. With 660,000 rep, however, there was a marked decrease in heat resistance at 66-68°C compared to that at 20° or -78°C. Additional investigation by Licciardello and Nickerson (1963)[12] determined that B. subtilis was less sensitized to 90°C heat than C. sporogenes when the spores of these organisms had been irradiated previously in phosphate buffer at room temperature. An irradiation dose of 800,000 rep prior to heating reduced the D value* of C. sporogenes by 90 percent whereas with B. subtilis about a 1,200,000 rep treatment was needed to achieve the same result.

Koesterer (1964)[13] investigated the sequential and simultaneous effects of gamma radiation and heat to determine what sequence of these agents was effective and to what degree. He exposed spores of B. subtilis var. niger and Bacillus coagulans on filter paper strips to dry heat treatment at 243°F prior to, simultaneously with, and following gamma irradiation at 2×10^4 rads per hour. The results showed that simultaneous treatment with dry heat and radiation was one of the most effective sequences.

It would be impossible to summarize the proceeding work in any quantitative way because of the diversity of experimental conditions. Although most investigators observed complementary effects when radiation and heat were used, most work was outside the range of parameters in which we were interested. Temperatures

*A "D" value is the time required for a given microbial population to be reduced by 90 percent or one log in population at a given temperature. The D value can also be the radiation dose required to reduce a given population by one log.

considered were very high or very low and radiation exposures were generally quite high when considering spacecraft sterilization. Also, we needed to define the shape of survivor curves through a greater range than is commonly considered.

Materials and Methods

Preparation of Samples

The stock of B. subtilis var. niger used in the experiments was obtained from the United States Biological Center, Fort Detrick, Maryland, as a dry powder. The spores were first suspended in 95-percent ethanol and then ultrasonically treated for separation of vegetative material from the spores. The spore stock was then cleaned by centrifugation and resuspension until a uniform button of spores was obtained. These were inspected microscopically and resuspended in ethanol to a concentration of 10^7 per ml. The suspension was then stored at 4°C.

Prior to experimental use, the spore suspension was subjected to ultrasonic treatment for 2 minutes. Experiment samples were prepared by pipeting 0.1 ml of the suspension onto 0.0015-inch thick x 1.18-inch diameter disks cut from biological grade aluminum foil. Samples were desiccated over Drierite in vacuo for approximately 1 hour and then assembled with a cover foil between two 0.025-inch aluminum strips, with four samples per strip. This assembly operation was completed in air at a relative humidity of less than 5 percent. Samples were again placed in a desiccator in vacuo for 15 hours before exposure to sterilization environment.

Exposure Methods

The radiation environment was provided by the Gamma Irradiation Facility. The facility consists of two hot cells[14] above a pool. Self-contained handling equipment permits sources to be readily introduced and removed from the cells. Provisions are included for visual, physical, and electrical access as well as the necessary safety controls. Each cell is 7 feet deep, 8 feet wide, and 8-1/2 feet high. The cobalt-60 sources located in the corners of each cell provide gamma dose rates[15]

of approximately 1 x 10^6 rads/hr (H_2O) to less than 4 x 10^3 rads/hr depending on the positioning of the sample within the cells.

The thermal environment was provided by a small circulating air oven that had been modified to accept aluminum sample strips in a rail arrangement as shown in Figure 1. Temperature was recorded and controlled to within ±1/4°C over the entire test period. The same oven was used for thermoradiation experiments by locating the oven in the hot cell at the appropriate distance from the cobalt-60 source to receive the desired dose rates. The solid state temperature control and other instrumentation were located outside the hot cell. The thin oven walls and absence of structural members in the oven wall minimized shadowing of samples from gamma rays. Silver phosphate dosimeters mounted on each of the sample strips verified computed dose rates. Moisture control was provided by the system shown in Figure 2. Room air was dried in one leg of the system to less than 1 percent RH by passing it through a desiccant bed of Drierite which was continuously monitored by a LiCl sensor and recorded. In a second leg of the system, air was heated to a temperature slightly above room ambient, saturated, and then cooled to room temperature, with the excess moisture being condensed into a trap. These two flows were then mixed to provide a continuous flow with controlled relative humidity. The relative humidity of this mixture was measured and recorded and the air then admitted to the oven at a rate of 1 cfm. This rate provided one air change per minute, minimizing effects of sample moisture and oven leakage. Air taken from the oven was cooled to room temperature and its relative humidity was continuously measured and recorded.

The LiCl sensors used were the narrow range type covering an RH range of 5 to 15 percent with specific range sensors. Sensors and multipoint strip chart recorders were calibrated as a system (in Sandia's Primary Standards Laboratory) before and after each series; as a result, RH measurements were accurate to within ±1 percent.

Recovery Methods

After being removed from the oven, each sample strip was wrapped in sterile foil and returned to the clean room facilities for recovery. About 30 minutes were required to transport irradiated samples from the remote reactor area to the

Figure 1. Circulating Air Oven

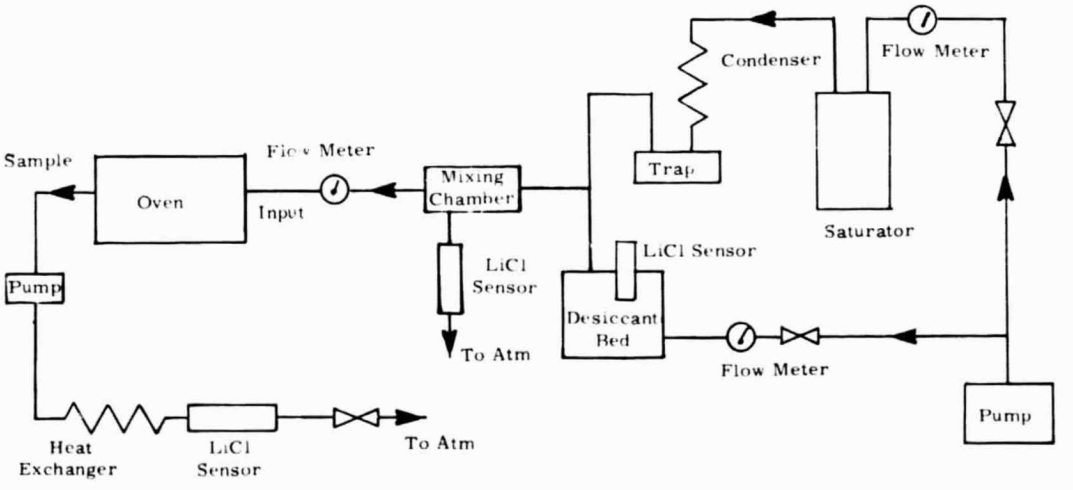

Figure 2. Humidity Control Schematic

Class 100 clean room facilities where all microbiological preparation and recovery were performed.

Sample strips, each of which represented a single data point, were then disassembled. The four separate samples within the strips were each placed in a 50-ml beaker containing 10 ml of sterile, 0.1 percent Tween 80 water. Samples were then ultrasonically treated for 2 minutes to remove the organisms from the foils. Additional ten-fold serial dilutions were prepared as required and plated out in duplicate on Trypticase Soy Agar. Plate counts were made after 72 hours incubation at 35°C.

Results

Thermoradiation at Various Temperatures

A series of experiments were performed using gamma radiation alone in order to develop a base line for that single environment. B. subtilis spores were exposed to gamma radiation at room temperature (21°C) and 30-percent room ambient RH. It was found that an approximate dose of 150 krads was required per log reduction in population; results of two runs are shown in Figure 3.

Next, a series of experiments were performed where environmental parameters were individually varied. Effects of these variations were then analyzed. First, the effect of dry heat alone was assessed at 125°C. The curve illustrating the survivors after this treatment is labeled 125°C in Figure 4. Another series samples were given a 150-krad dose of radiation at room temperature and then exposed to heat treatment at 125°C. The survivor curve for the preirradiated spores is also shown in Figure 4. Finally, spores were exposed to the simultaneous application of dry heat and radiation (again refer to Figure 4). It is interesting to note that the curve representing simultaneous radiation and heat treatment at 125°C is about three logs below the 125°C curve. If the environments were only additive, the simultaneous curve should be less than a log down from the dry heat curve since the radiation dose at this point was only 100 krads.

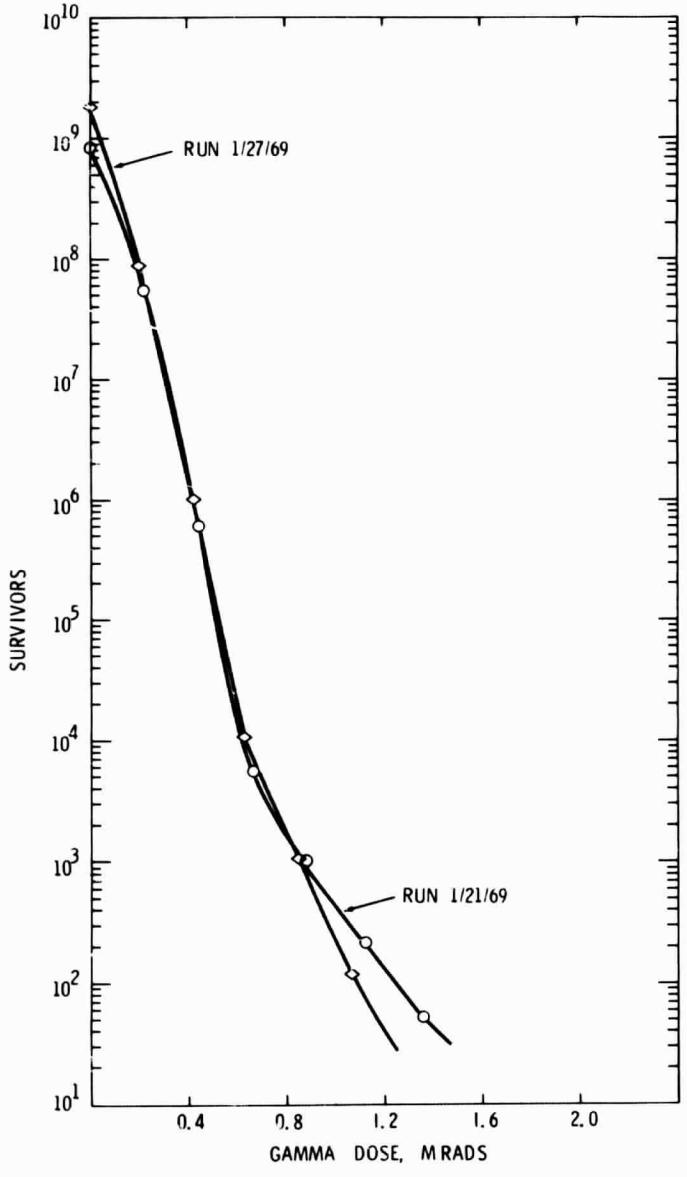

Figure 3. Radiation Sterilization Composite

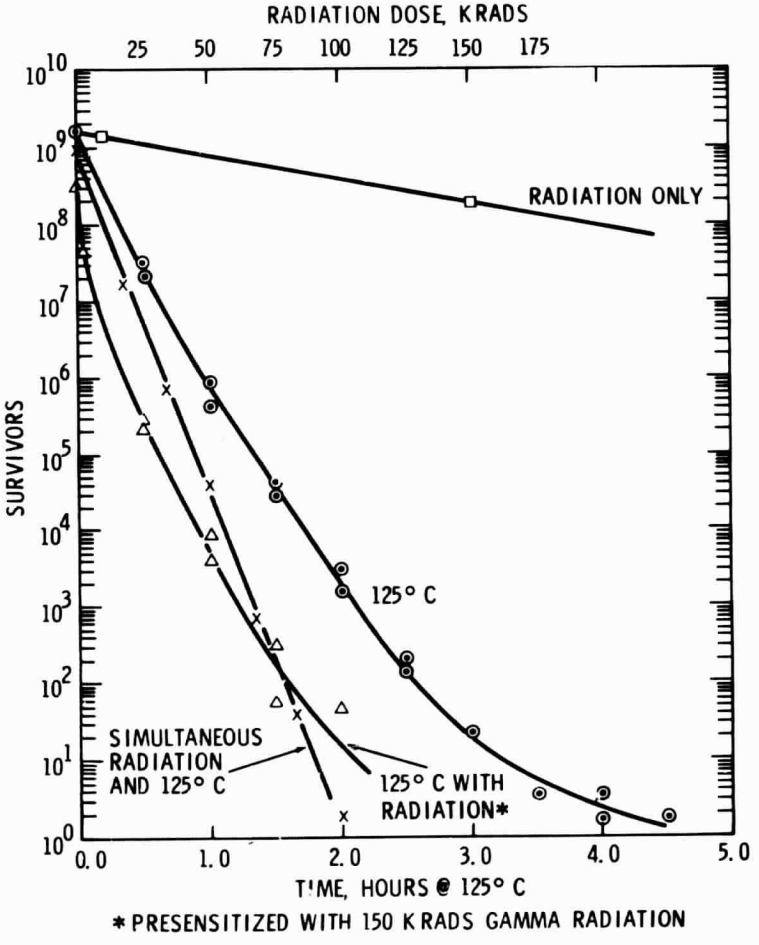

Figure 4. Comparison of Radiation/Dry Heat Sterilization of Bacillus subtilis

A second series of experiments were performed with a base temperature of 105°C. Results are shown in Figure 5. It should be noted that the survivor curve for radiation alone is slightly steeper than the one shown in Figure 4. It is suspected that the higher lethal effect was caused by the greater number of interruptions in the radiation application as samples were removed. The remaining curves on Figure 5 are similar to the 125°C curves (Figure 4) in that simultaneous application of heat and radiation is more effective than sequential application. The survivor curve representing simultaneous heat and radiation is six logs down from the dry heat curve with a gamma dose of less than 100 krads. One can conclude that a synergistic effect of approximately five logs has been experienced.

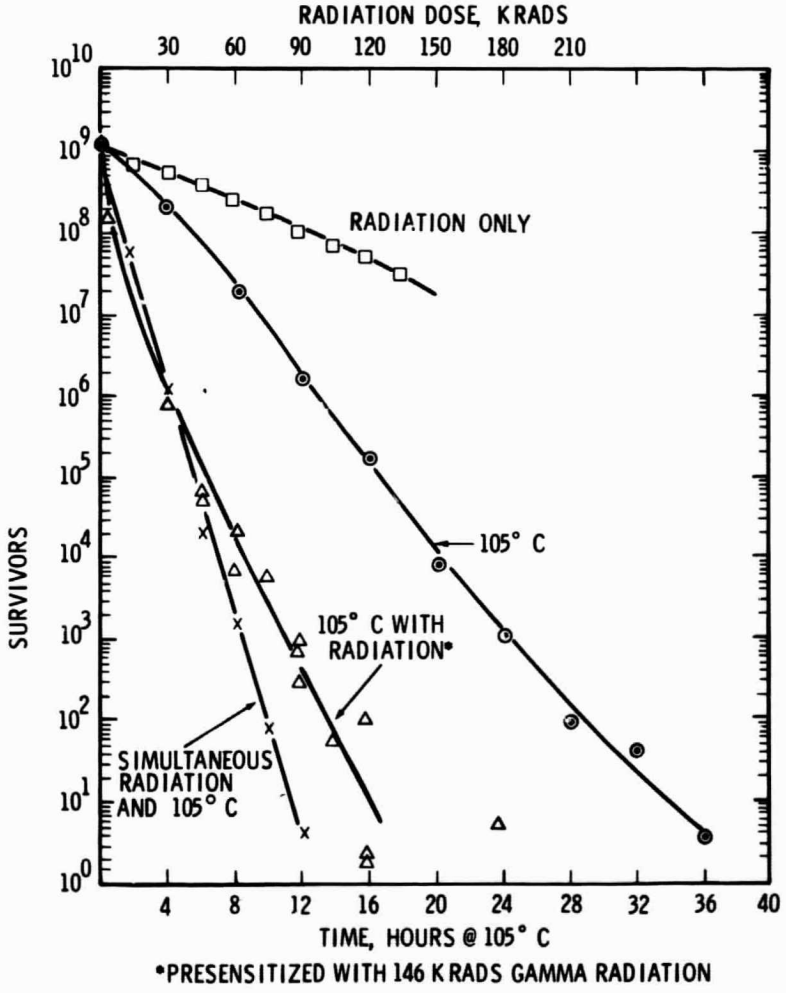

Figure 5. Comparison of Radiation/Dry Heat Sterilization of Bacillus subtilis

Next in the series were experiments at 100°C; these results are shown in Figure 6. Again, about the same degree of synergism was experienced; however, in this case the radiation rate was slightly higher, resulting in a total dose of 180 krads at 24 hours.

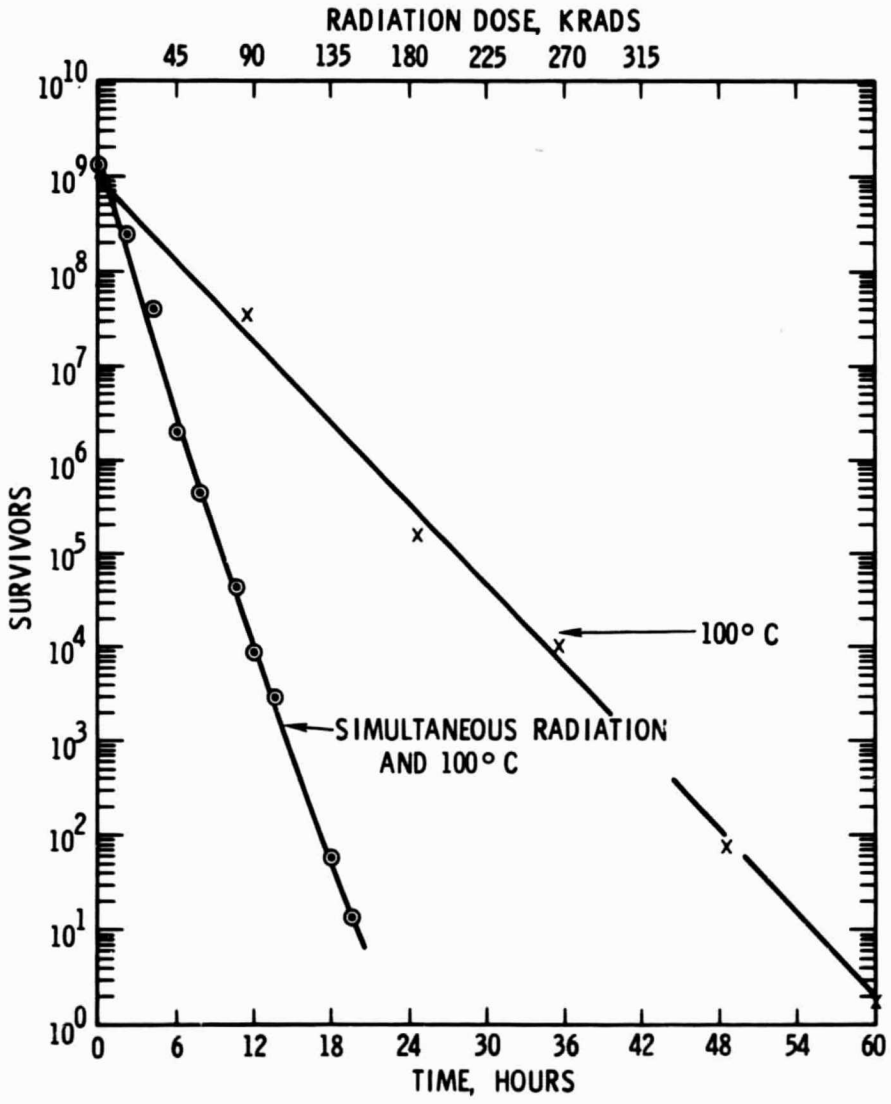

Figure 6. Comparison of Radiation/Dry Heat Sterilization of Bacillus subtilis

Results of all of these experiments are summarized in Figure 7. This illustration shows the reduction in dry-heat D value resulting from the combined application of heat and radiation. In each case the open bar to the left of zero represents the D value for heat alone, and to the right of zero the D value for radiation alone. The cross-hatched area represents the total combined thermoradiation treatment needed for an equivalent one-log decrease in population. In other words, at 125°C the D value was reduced from 22 minutes with dry heat alone to 12 minutes for simultaneous conditions with a radiation dose of 10 krads applied during that 12-minute period. At 105°C the D value was reduced from 4.5 hours to 1.5 hours with a gamma dose of 11 krads. At 100°C the D value was reduced from 7 hours to 2.5 hours with a gamma dose of 19 krads.

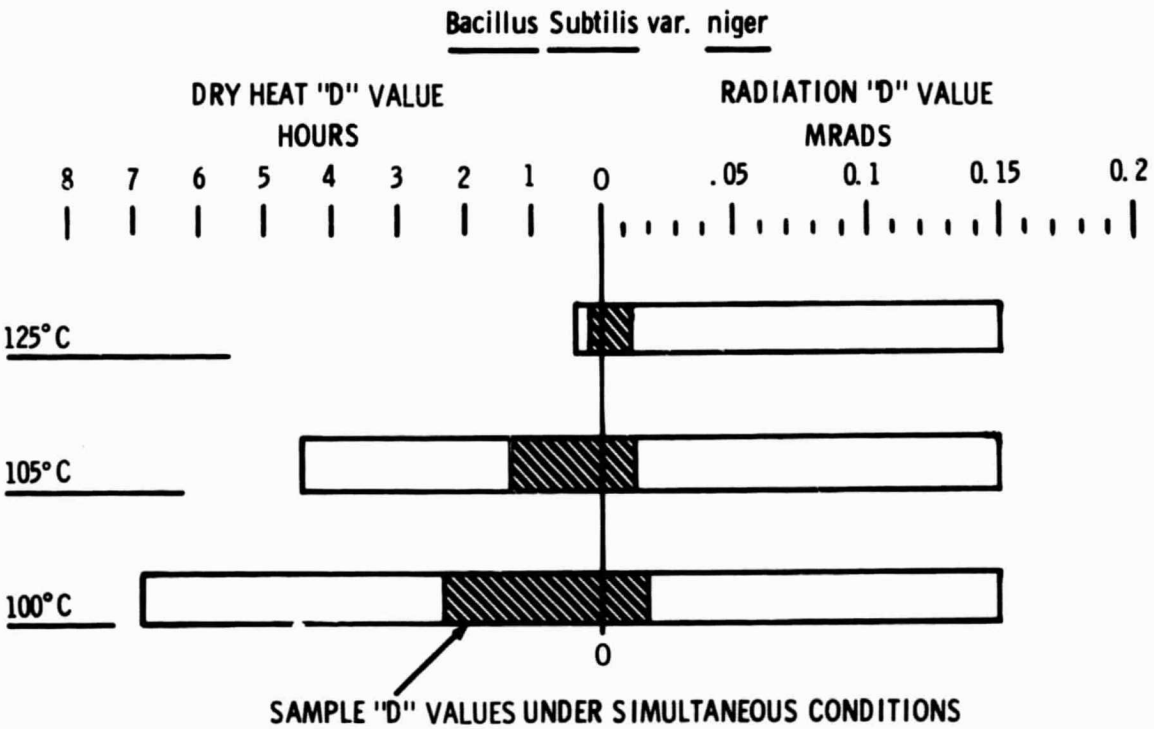

Figure 7. Experiment Results

Experimental Sensitivity

After this initial investigation, we decided to determine the sensitivity of thermoradiation to several additional environmental parameters that could perhaps effect experimentation. The first parameter was film thickness of organisms.

We started with an initial population of 10^9 B. subtilis spores per sample, or roughly 10^9 spores per square inch. This was done to define the shape of the survivor curve for at least seven logs before the actual plate counts become sparse. In these initial thermoradiation experiments the survivor curves in all cases are first order, exhibiting no tailing as is sometimes found in dry heat experiments. Thus, we were able to reduce the initial population to the more frequently used loading of 10^6 spores/in.^{2}. The survivor curves for the two initial populations are shown in Figure 8. Comparing the two curves, one can conclude that in the lower and more realistic initial loadings of 10^6 organisms, thermoradiation is more effective.

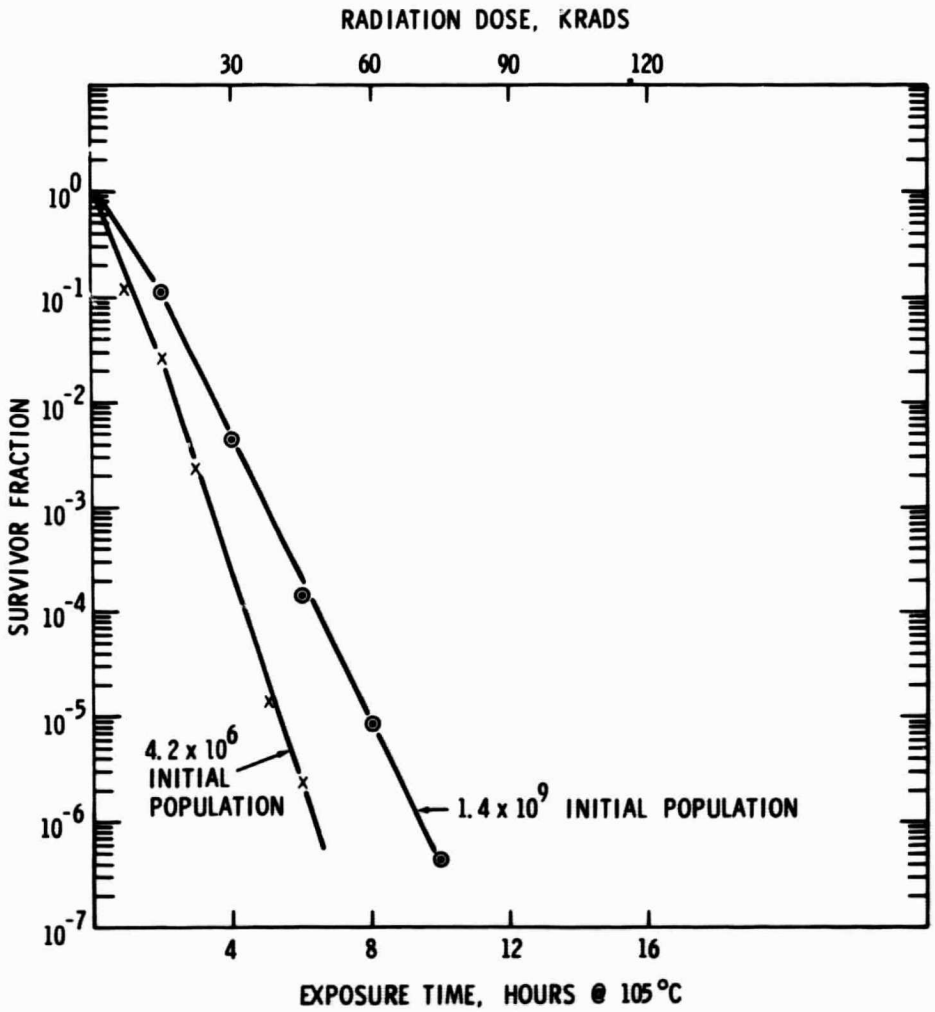

Figure 8. Comparison of Radiation/Dry Heat Sterilization of Bacillus subtilis with Varied Initial Loading

The second parameter to be investigated was relative humidity. The effect of changing relative humidity within the laboratory is known to affect experimentation, particularly in dry heat studies. Using the humidity control equipment previously described, we performed a series of dry heat experiments at relative humidity extremes which most certainly bracket the 35-percent RH level typical of our laboratory environments. The extremes chosen were 20 to 60 percent RH at room temperature. It is interesting to note that this spread in humidity represents a change to only 0.5 and 1.5 percent RH, respectively, when the air is heated in the oven to 100°C (Figure 9).

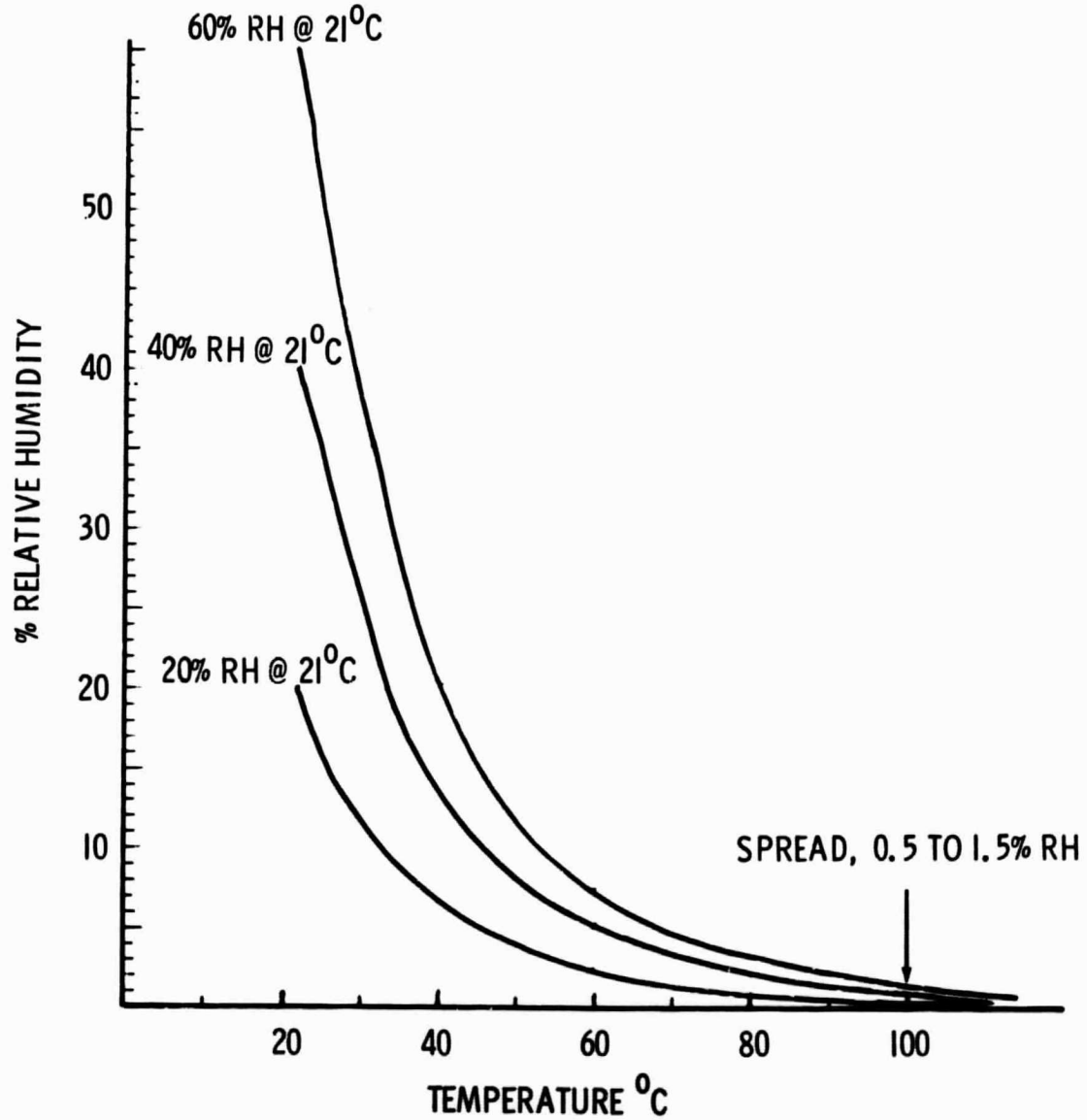

Figure 9. Lines of Constant Moisture

Dry heat survivor curves for B. subtilis under differing relative humidity conditions are compared in Figure 10. There is indeed an effect on the lethality of dry heat when the room ambient conditions are varied from 20 to 60 percent RH. The 105°C dry heat D value changes from 2.3 hours at 20 percent RH to 5.3 hours at 60 percent RH.

The same experiments were next performed using heat and gamma radiation (see Figure 11). It was interesting to find that, at least within the range of dose rate and temperature that we have been using, there seems to be little effect from changing ambient relative humidity during thermoradiation exposure. This can be explained by the differing effects moisture has on the lethality of heat as compared to the effect it has on the lethality of ionizing radiation. In the dry heat comparison, Figure 10, it was shown that between 20 and 60 percent RH, B. subtilis becomes more sensitive to heat at the lower moisture content. In the radiation environment, the removal of water from a system normally increases the resistance of the organisms concerned.[18]

Heat and Radiation Resistance of Other Organisms

To demonstrate the feasibility of thermoradiation sterilization as a technique for spacecraft sterilization, the effectiveness of the technique on bacteria other than B. subtilis must be examined. Figure 12 is a comparison of other organic D values for dry heat and radiation, using average published values. These organisms were chosen because of their wide use as sterilization controls. B. subtilis var. niger is used as a sterilization control in dry heat studies for NASA. Bacillus stearothermophilus is a U.S. Industrial control, used because of its high resistance to moist heat. Bacillus pumilus is a British sterilization control, and Streptococcus faecium is the Danish control used in radiation sterilization. It is clear from Figure 12 that there are other organisms much more resistant to heat and more resistant to gamma radiation than is B. subtilis.

We have thus far completed a single series of experiments on B. pumilus. The results of this experiment were consistent with the demonstrated effectiveness of thermoradiation on B. subtilis. The D value at 105°C was reduced from 2.7 hours for dry heat alone to 1 hour for simultaneously applied gamma radiation (7 krads) and dry heat.

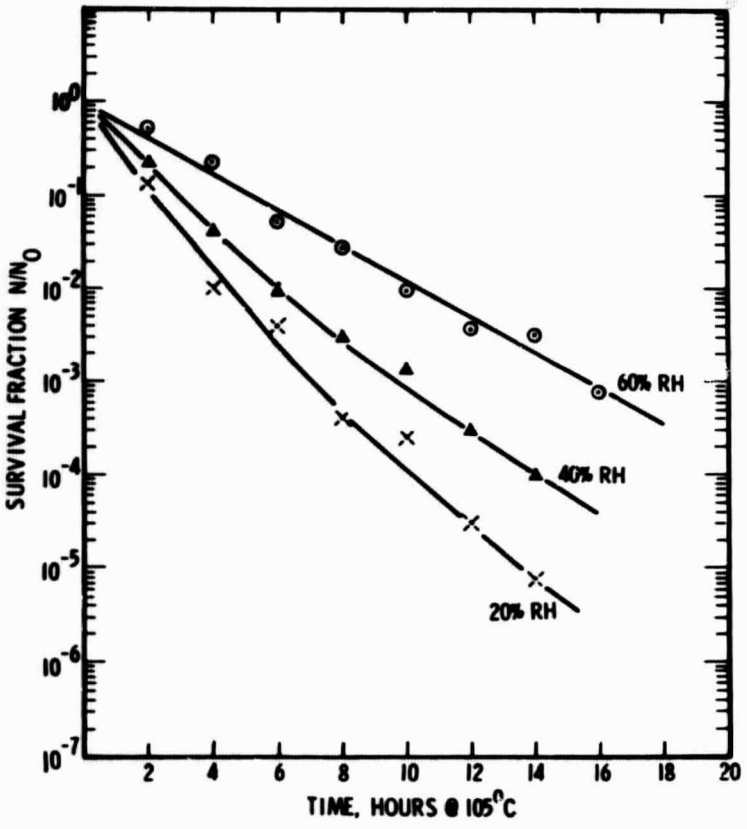

Figure 10. Comparison of Dry Heat with Variable Room Ambient Relative Humidity

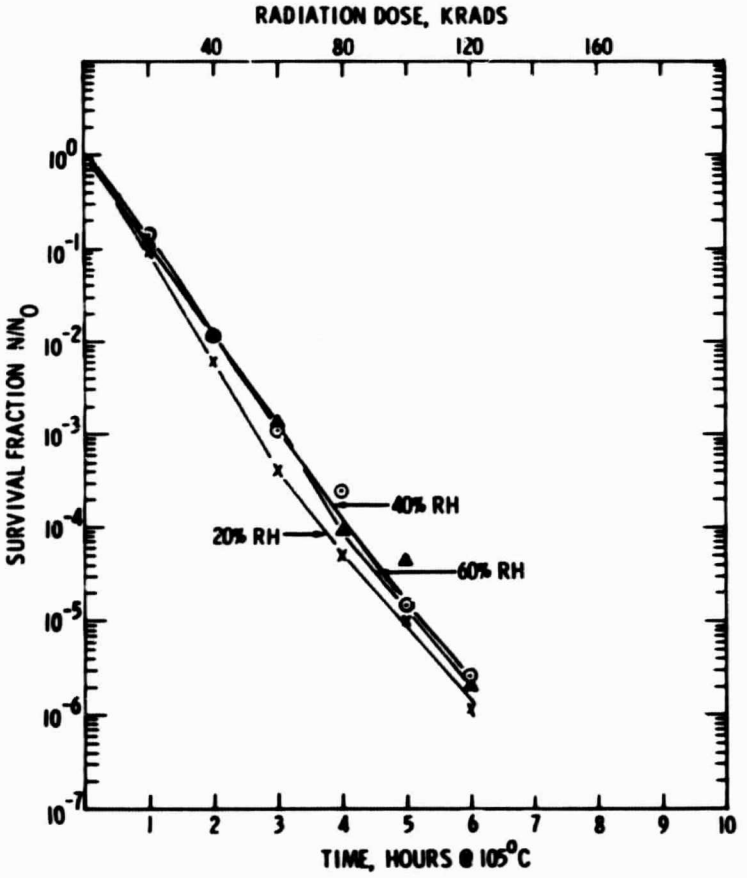

Figure 11. Comparison of Simultaneous Radiation/Dry Heat with Variable Room Ambient Relative Humidity

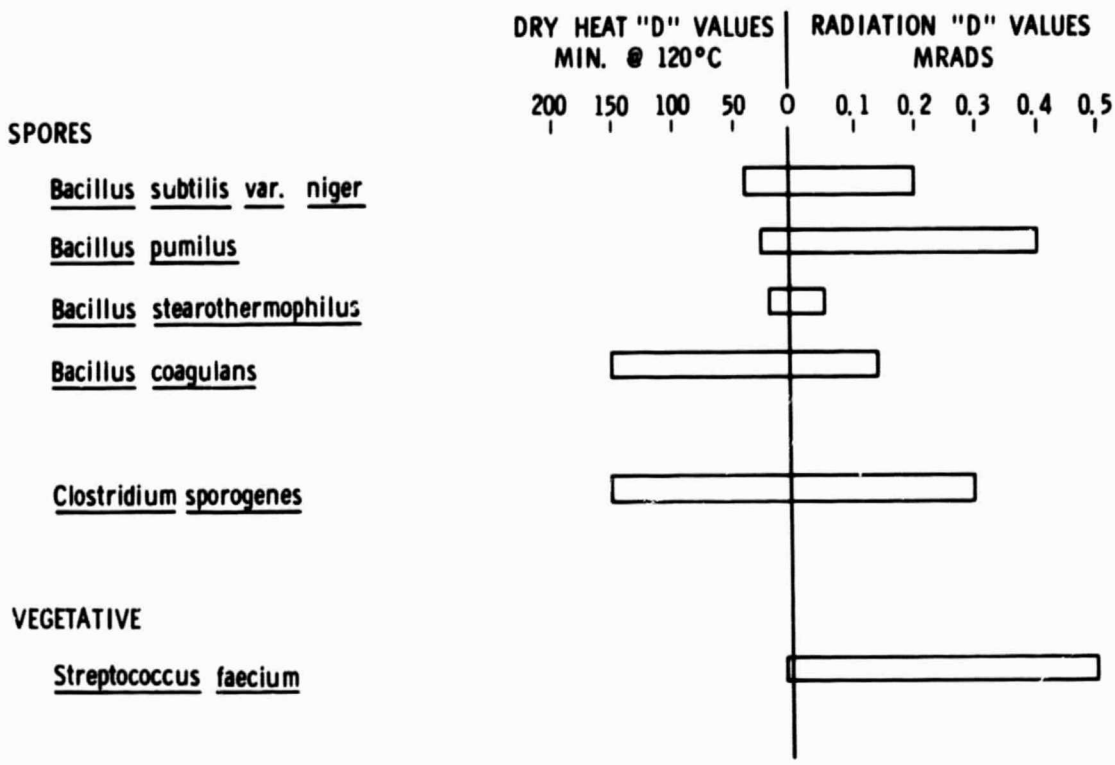

Figure 12. Comparative Heat and Radiation D Values

Conclusions

Although this initial phase of the study has not been done in depth, it has demonstrated that bacterial sterilization, using B. subtilis and B. pumilus can be accomplished at relatively low temperatures and low levels of radiation. These low levels of exposure, enhanced by rather substantial synergistic effects, suggests a fascinating potential for thermoradiation sterilization in many areas: pharmaceuticals and medical products, for example, but, in particular, spacecraft applications. We have also satisfied ourselves that the synergism is not a technique-induced or other artifact resulting from materials providing chemical sterilization in the radiation environment. It is not caused by unusual spore film thicknesses nor is the synergism caused by changing relative humidity effects.

We feel that at this time the purpose of the initial phase of this study has been satisfied and continuation of the study in depth is indicated.

Continuation of Work

The desirability of thermoradiation as a replacement for dry heat sterilization of spacecraft must yet be fully demonstrated. In the coming year we plan to further explore the effectiveness of thermoradiation sterilization on B. subtilis var. niger and other appropriate organisms. We hope to optimize and demonstrate sterilization cycles at various temperatures and radiation exposures. The inactivation mechanism or mechanisms are also being studied to develop an understanding of the processes involved. Such understanding would substantially assist optimization of a sterilization cycle.

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