CHAPTER 3
THE APOLLO 16 MICROBIAL RESPONSE TO SPACE ENVIRONMENT EXPERIMENT

by

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Introduction

Microorganisms have been subjected to a large variety of space flight conditions on many United States and Soviet missions including Sputnik 4–6, Vostok 1–6, Voskhod 1 and 2, Cosmos 110, Nerv 1, Discoverer 18, Gemini 9, 10, and 13, Agena 8, and Biosatellite 2. This considerable number of flights carried a large array of viruses, bacteria, and fungi which were exposed to many different space flight conditions.

Most of these past studies were concerned with establishing the now accepted principle that microbes can survive in the harsh space environment. However, during the conduct of these viability studies certain anomalies were noticed which suggested that the survival of some microbes was enhanced, whereas others were adversely affected by the space environment. For example, aqueous suspensions of spores from members of the genus *Streptomyces* (*Actinomyces* in the Soviet Union) demonstrated quite different results following exposure to space flight conditions aboard the third, fourth, and fifth Soviet satellites (Glembotskiy et al., 1962). The space flight conditions reportedly

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increased the incidence of spore germinations of Strain 2577 of *S. erythreus* by about six times that of the ground controls, whereas the viability of Strain 8594 decreased sharply. These examples are typical of past survival studies where results are quite evenly divided among those which report synergism, antagonism, or no relationship at all between space flight and microbial viability (de Serres, 1969; Glembotskiy et al., 1962; Kovyazin et al., 1962; Lorenz, 1968; Mattoni, 1968; & Parfenov, 1967). Many previous studies were hindered by technical constraints, mission anomalies, or the inability to provide meaningful controls. As a result, and in spite of the best effort of the investigators, equivocal results were often produced. One of the objectives of the present experiment was to take advantage of the considerable array of past experimentation, overcome as many equivocating obstacles as possible, and help to establish a meaningful relationship between space flight and the viability of several different microbial systems.

A few of the more recent United States and Soviet microbiology studies have investigated the effect of space flight on other parameters in addition to viability. Generally, these studies have involved genetic changes, and as with the survival studies, they have produced variable results (Antipov, 1967; Antipov et al., 1969; de Serres, 1969; de Serres & Webber, 1968; de Serres et al., 1969; Jenkins, 1968; Mattoni, 1968; Parfenov, 1967; & Zhukov-Verezhnikov et al., 1963). However, the combined results of these studies suggest the possibility that the conditions of space flight influence microbial genetic alterations (Townes, 1970). The "Microbial Response to Space Environment" experiment was designed to evaluate this effect as well as to determine the survivability of microorganism species.

General Experiment Design

From the multitude of microbial species and challenge systems available, the experiment system outlined in table 1 was established. This experiment system comprised a variety of species, each of which may be considered to be a model system for evaluation of some medically important activity. Investigators were invited to study those phenomena within their area of expertise, and to conduct critical investigations in their own laboratories. This method allowed a large number of individual studies to be conducted in a coordinated manner, and permitted a variety of species to be housed within a single piece of flight hardware. Each investigator selected a test system which was nonpathogenic to man (to avoid possible contamination of the crew), was well characterized relative to the phenomenon to be studied, lent itself well to simple and rapid screening tests, and was compatible with the unique environment of the flight hardware.

In order to allow for dose-response studies and comparative investigations, certain variables were provided within the flight hardware. Microbes could either be suspended in 50 microliters of fluid or could be dried on a suitable carrier. Some of the microbes were exposed to the vacuum of space whereas others were retained at one atmosphere. As detailed genetic studies required exposure to a mutagenic source, provisions were made to expose test systems to the full light of space or to components of the solar ultraviolet spectrum at peak wavelengths of 254 nm, 280 nm, and 300 nm. An optical filtering system was provided to control the total radiant energy reaching exposed test systems

from a minimum of 4×10^1 ergs cm^{-2} to a maximum of 8×10^8 ergs cm^{-2}. The use of ambient solar radiant energy as the mutagen necessitated close monitoring of this factor. Photographic emulsion and a modification of the potassium ferrioxalate system of Wrighton and Witz (1972) were used to record the amount of radiant energy which actually reached selected test systems (table 2). The possible mutagenic activity of galactic radiation necessitated the inclusion of lithium fluoride thermoluminescent dosimeters and a package of passive nuclear track detectors capable of recording high-energy multicharged particles (table 2).

Table 1

Biological Components

Phenomenon Studied	Assay System	Microorganism	Investigator
Lipolytic α toxin production	Lytic zone on agar	*Bacillus thuringiensis*	R. T. Wrenn, W. L. Ellis, Northrop Services, Inc., Houston, Texas; G. R. Taylor, R. C. Simmonds, NASA Manned Spacecraft Center, Houston, Texas; A. M. Heimpel, U. S. Dept. of Agriculture, Beltsville, Maryland
Deforming β toxin production	*Sarcina flava* and house fly		
Fatal δ toxin production	Silk worm and crystal assay		
Infectivity	Mouse	*Nematospiroides dubius*	R. A. Long, W. L. Ellis, Northrop Services, Inc., Houston, Texas; G. R. Taylor, NASA Manned Spacecraft Center, Houston, Texas
Hemorrhagic factor production	Guinea pig and hemoglobin	*Aeromonas proteolytica*	B. G. Foster, D. O. Lovett, Texas A. & M. University, College Station, Texas
Hemolytic enzyme production	Human erythrocytes		
Genome alteration	Spore production	*Bacillus subtilis* spores, strains HA 101 HA 101 (59) F	J. Spizizen, J. E. Isherwood, Scripps Clinic and Research Foundation, La Jolla, California
UV and vacuum sensitivity	Colony formation	*Bacillus subtilis* spores, strain 168	H. Bücker, G. Horneck, H. Wollenhaupt, University of Frankfurt, Germany
Bacteria phage infectivity	Host lysis	*Escherichia coli* (T-7 phage)	J. Spizizen, J. E. Isherwood, Scripps Clinic and Research Foundation, La Jolla, California
Cellulolytic activity	Cloth fibers	*Chaetomium globosum*	P. A. Volz, Y. C. Hsu, D. E. Jerger, J. L. Hiser, J. M. Veselenak, Eastern Michigan University, Ypsilanti, Michigan
Animal tissue invasion	Human hair	*Trichophyton terrestre*	
Drug sensitivity	Antibiotic sensitivity in agar	*Rhodotorula rubra* *Saccharomyces cerivisiae*	

Table 2

Dosimetry Components

Measurement	Monitor Used	Assay Systems	Investigator
High-energy multicharged particles	Passive nuclear track detectors	Lexan Cellulose nitrate Photographic emulsion Silver chloride	E. V. Benton University of San Francisco San Francisco, California
Ultraviolet light	Passive dosimeters	Potassium ferrioxalate actinometry Photographic emulsion	M. B. Parson, R. A. Long, W. Ellis Northrop Services, Inc. Houston, Texas G. R. Taylor NASA Manned Spacecraft Center Houston, Texas
Penetration of galactic irradiation	Thermo-luminescent dosimeters	Lithium fluoride	J. V. Bailey NASA Manned Spacecraft Center Houston, Texas R. A. English, R. D. Brown Kelsey-Seybold Clinic Houston, Texas

These latter studies were conducted in a manner that allowed for direct correlation with similar readings obtained from the BIOSTACK experiment, the Apollo crew personnel radiation dosimeters, and the Apollo Light Flash Moving Emulsion Detector (ALFMED), all of which were used in the Apollo 16 Command Module.

Description of the Flight Hardware

Each biological test sample, containing 10^2 to 10^6 living cells as appropriate, was housed in a chamber (cuvette) 5 mm on a side, composed of Kel-f plastic with a quartz window (figure 1). There were three types of these chambers, one of which was designed to contain 50 microliters of fluid. This type possessed, on the side opposite the quartz window, a fill port which was sealed with Shelwax 500 after filling with the test solution. The cuvette body was designed to have a seven-degree internal slope to prevent possible shadowing of the organisms.

The other two cuvette types were both designed to retain biological test systems which had been deposited on Millipore filter chips with a mean pore size of 0.45 microns. The only difference between these latter two types was that one was vented to the outside, thus allowing for exposure of the contents to the vacuum of space (figure 1).

All loaded cuvettes which were to be exposed to UV irradiation were placed beneath neutral density filters which were situated under bandpass filters. This optical filter combination respectively controlled the amount and the wavelength of light reaching the microbial systems (figure 2). Cuvettes and optical filters were placed in trays (figure 3) which were mounted in a hardware case which measured 11.4 x 11.4 x 24.5 cm. The

flight hardware (figure 4), designated the Microbial Ecology Evaluation Device (MEED), contained 798 cuvettes with biological test systems, 140 neutral density filters, 28 bandpass filters, eight recording thermometers, one high-energy multicharged particle dosimeter, 64 potassium ferrioxalate actinometry cuvettes, 44 photographic film cuvettes, and 18 thermoluminescent dosimetry cuvettes. The flight hardware was placed within a stowage bag which helped absorb the launch vibrations and provided additional thermal insulation. The bag was made from nonflammable Beta cloth and nonflammable Fluorel sponge foam.

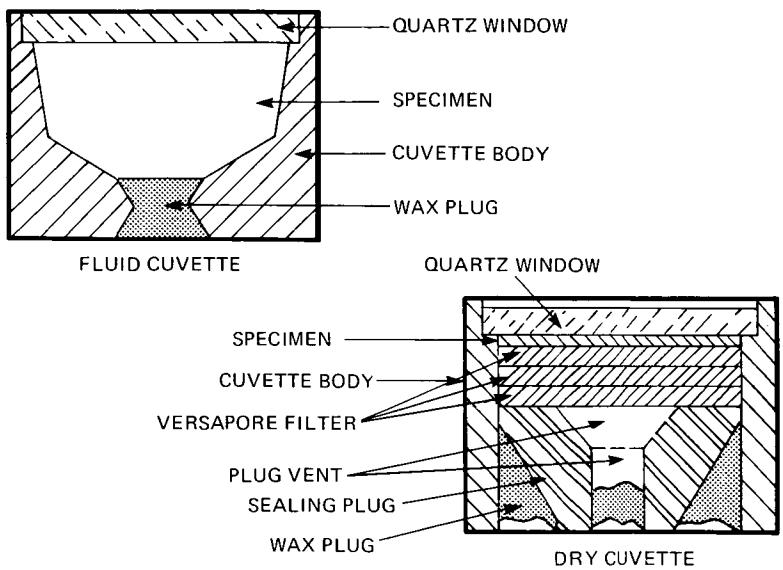

Figure 1. Biological test sample cuvette design.

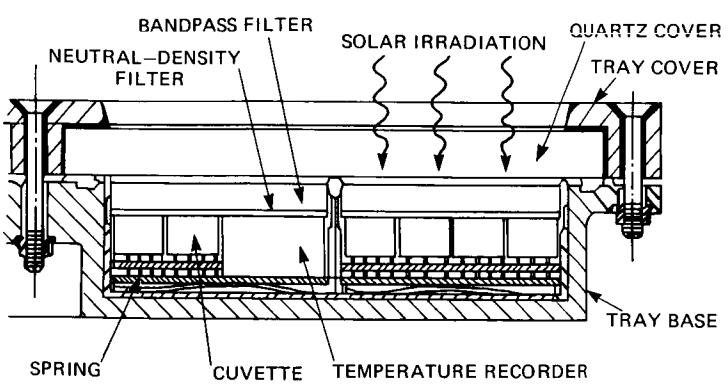

Figure 2. Optical filter configuration.

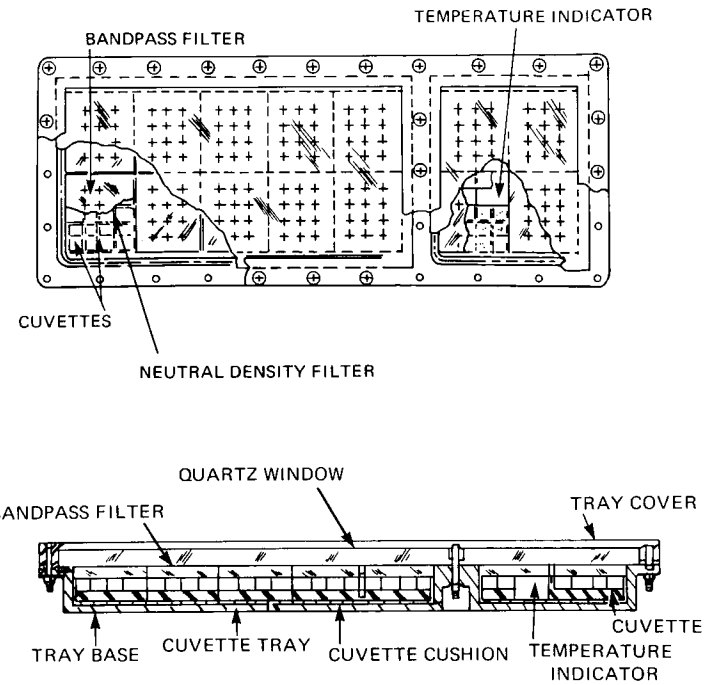

Figure 3. Details of MEED tray interior.

Deployment of the Microbial Ecology Evaluation Device

During the extravehicular activity phase of the Apollo 16 transearth coast, the MEED hardware was removed from its protective stowage bag while in the crew compartment and affixed to the distal end of the television boom which was then attached to the handle of the opened hatch door (figure 4). The procedure of deploying the MEED hardware by an Apollo 16 astronaut is shown in figure 5.

A small attitude adjustment of the Command Module was required to place the appropriate surface of the MEED directly perpendicular to the rays of the sun. This was indicated by a solar positioning device incorporated into the exterior surface of the MEED. After attaining the proper attitude, the MEED was opened so that the biological test systems and actinometers were exposed to the direct rays of the sun. After exactly ten minutes of such exposure the device was closed, removed from the television boom, and replaced in its protective bag for transport back to the Johnson Space Center.

Design of Individual Test Systems

Aeromonas proteolytica

This microorganism was selected for studying the effects of solar irradiation and space flight conditions on the production of extracellular enzymes because it produces an

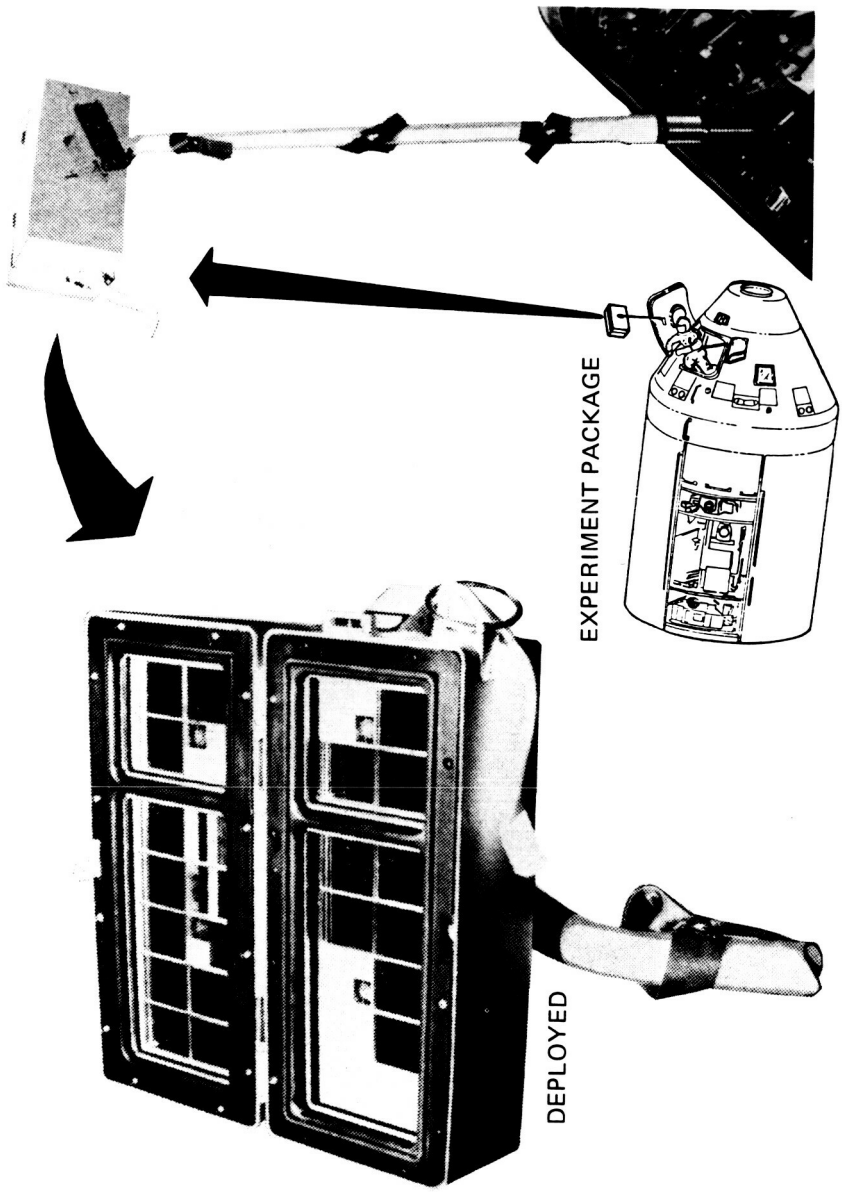

Figure 4. Hardware for the Microbial Ecology Evaluation Device.

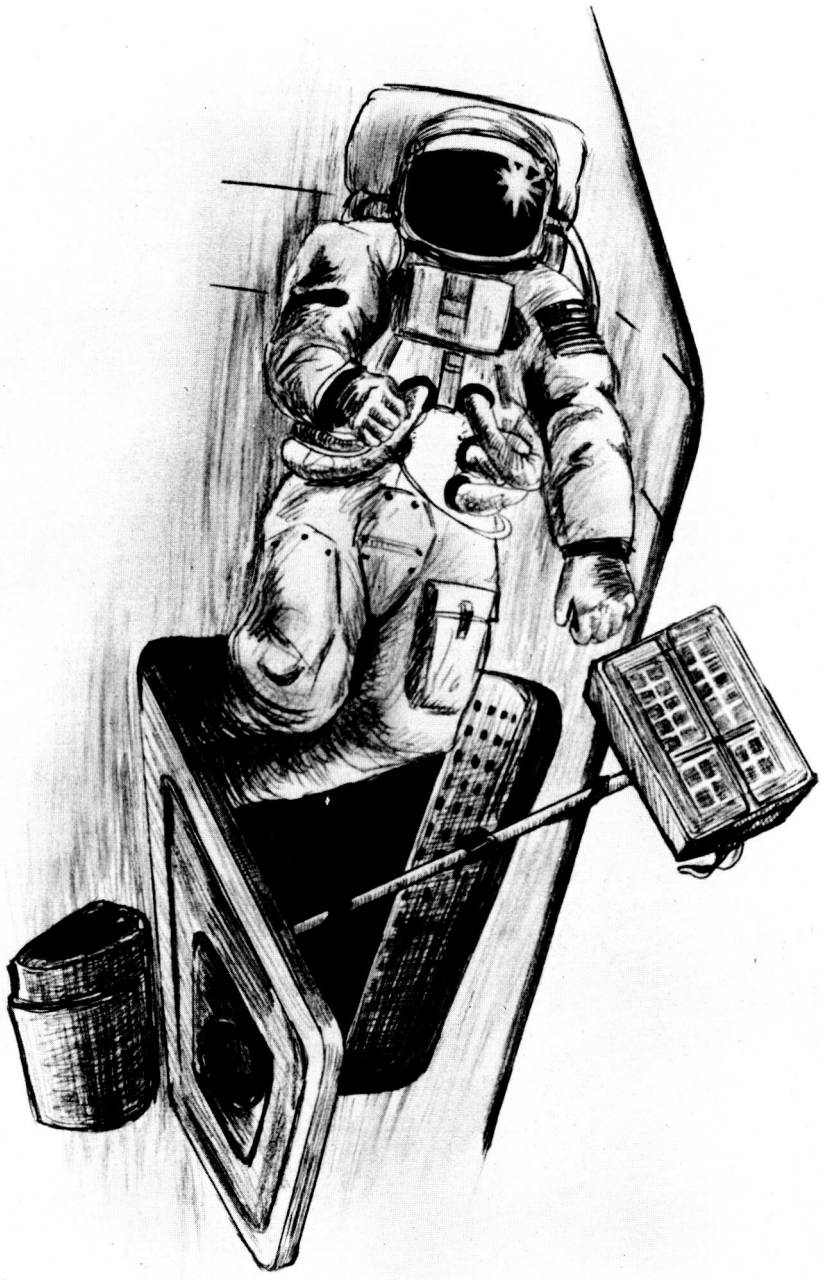

Figure 5. Artist's conception of inflight deployment of MEED hardware by an astronaut.

endopeptidase which can cause intracutaneous hemorrhage and necrosis in laboratory animals (Foster, 1972), and a hemolysin which is elaborated into the culture fluid and has the ability to hemolyse human erythrocytes (Foster, 1972). This microbe was retained in fluid suspensions and exposed to solar ultraviolet irradiation at peak wavelengths of 254 nm, 280 nm, and 300 nm. In addition to survival evaluations, cells recovered from the flight hardware were quantitatively tested for alterations in toxin production. Postflight analysis of retrieved cells indicated that there was no significant difference between the survival rates of inflight and ground-based control.

Bacillus subtilis

Different strains of this species were evaluated by two different groups as indicated in table 1. Spores of this species are generally highly resistant to harsh environments and were therefore expected to yield a high return of viable cells for detailed genetic analyses.

The manner in which spores of Strain 168 survive when exposed to one or more factors of space has been critically studied in simulation experiments (Horneck et al., 1971) as well as in the BIOSTACK experiment which was flown on Apollo 16 and 17. For the present study, spores were exposed to space vacuum and solar ultraviolet irradiation at a peak wavelength of 254 nm to determine the influence of these space factors on their survival evaluated in terms of colony-forming ability. The combined action of space vacuum and solar ultraviolet irradiation at a peak wavelength of 254 nm resulted in greater loss of viability than was observed in ground-based studies. Space vacuum alone did not cause a decrease in survival of predried spores, indicating that air-dried spores may survive exposure to space vacuum if shielded against solar irradiation. The additional environmental factors of space flight did not measurably influence the viability and irradiation response of spores of Strain 168.

Another investigative group exposed *Bacillus subtilis* spores of Strains HA 101 and HA 101 (59) F to the space flight environment both in aqueous suspensions and in dry layers, as outlined in table 1. These strains require three specific amino acids for growth which are used as identification and mutation detection markers. In addition, Strain HA 101 (59) F is defective in the ability to repair radiation damage (Gass et al., 1971), and is therefore highly susceptible to the damaging effects of ultraviolet irradiation. Generally, the lethal effects of irradiation at peak wavelengths of 254 and 280 nm were greater for dried spores than for those exposed to distilled water. Additionally, the repair-defective strain was more sensitive at both wavelengths of UV irradiation. As expected, survival rates for space flight-exposed spores did not differ significantly from analogous aliquots in the ground control units. Detailed genetic analyses are being performed to determine if any mutational effects of space flight were obtained.

Bacillus thuringiensis* var. *thuringiensis

This microorganism, which has widely been used as a biological insecticide, was selected for inclusion in this experiment because it produces three toxins which are active against biological systems and lends itself well to both rapid screening and critical *in vivo* analyses. The toxins include a lipolytic α-exotoxin which in some ways resembles the

Phospholipase C produced by *Clostridium perfringens*, a deforming β-exotoxin which is a nucleotide that is heat stable and kills insects at time of molt or pupation, and a crystalline δ-endotoxin which is a proteinaceous factor that destroys the midgut cells in many Lepidopterans, causing gut paralysis and eventual death (Heimpel, 1967).

As with the *B. subtilis* tested, there was no significant difference between the mean of *B. thuringiensis* survivors from the ground control, flight control, and vibration control units. Also, there was no significant difference between the means of survivors for any of the groups exposed to solar ultraviolet light in space. There was a significant difference ($p<0.01$) in the survival rate of those groups exposed to full sunlight in space when compared with the nonirradiated control groups. This indicates that the space-flown spores of this species were resistant to the levels of ultraviolet irradiation encountered in the test, but were sensitive to the full light of the sun. This follows previously established patterns obtained from ground-based studies (Cantwell & Franklin, 1966) and is not considered anomalous behavior.

Phage T7 for *Escherichia coli*

Survival studies of the T-7 bacteriophage of *Escherichia coli* were included in an attempt to relate the present experiment to the space flight-mediated effects reported by Soviet investigators for *E. coli* phages which were flown on numerous manned flights (Antipov, 1967; Hotchin, 1968; Lorenz, 1968; and Zhukov-Verezhnikov, 1968). Rather than the T-1 or K-12 (λ) phage commonly used in Soviet space flight studies, the simpler and more stable T-7 phage was chosen for this study in hopes that it would be more resistant to the rigors of space flight and therefore prove to be a better UV test subject. Postflight survival evaluations support this hypothesis because large losses in the non-UV exposed flight subjects (as compared to the ground controls) are not indicated. The lethal effect of inflight solar ultraviolet irradiation at a peak wavelength of 254 nm was considerably higher than ground-based controls which were exposed to the same levels of irradiation; however, the characteristic shape of the dose response curve was similar to the curve of the ground control data.

Nematospiroides dubius

This nematode was chosen for study largely because it is a complex multicellular organism which has been successfully cultured *in vitro* from the egg to the third stage infective larvae (Weinstein et al., 1969), is pathogenic to laboratory mice but not to humans, and is quite insensitive to the special holding conditions of the flight hardware.

A total of 2×10^5 ergs cm^{-2} of solar inflight ultraviolet irradiation at a peak wavelength of 254 nm was sufficient to completely inhibit ultimate infection in the murine host and subsequent maturity to adult worms. Therefore, the survival of space flight irradiated larvae was too low for further comparative studies.

Comparison of nonirradiated flight and ground control subjects revealed no differences in survival, infectivity in mice, formation of adults, or subsequent egg productions. There was, however, a significant decrease in egg viability within the group of adults which descended from flight control larvae that were exposed to the space flight environment (excluding vacuum) but received no solar ultraviolet irradiation. This was an

important observation since this control group was not purposefully exposed to any experimental stresses and was simply a "passenger" on the space flight.

Mycological Studies

Four different species of fungi (two filamentous fungi and two yeasts) were incorporated within the experiment package. Each of these species was carefully selected by the investigator (table 1) so that exhaustive postflight studies of medically important activities could be performed and compared to suitable ground-based controls.

Trichophyton terrestre was selected because it has the ability to attack human hair under laboratory conditions and has not been shown to be naturally infectious. The other filamentous fungus, *Chaetomium globosum*, was of special interest because of the cellulolytic activity it has demonstrated on cloth fibers, such as those which compose portions of the flight garments of the astronauts (Volz & Jerger, 1973). The two yeasts, *Rhodotorula rubra* and *Saccharomyces cerevisiae*, were included because they lend themselves well to drug sensitivity studies and other quantitative evaluations having medical importance. Analysis of postflight data indicate that in no case was there a significant difference between survival rates of static ground controls and other ground control aliquots which were subjected to simulated launch vibration. There were slight differences between survival rates of these two control series and the inflight controls (not irradiated) of three of the test species. The survival of flown *C. globosum*, *R. rubra*, and *S. cerevisiae* was slightly lower than corresponding ground controls. In addition, aliquots of *T. terrestre* and *S. cerevisiae* demonstrated some sensitivity to inflight solar ultraviolet irradiation when measured in terms of a loss of cell viability.

During the viability studies, selected isolates were recovered for ongoing postflight investigations. These additional studies include evaluations of hyphal growth dynamics and possible alterations in the chromosomal configuration of different filamentous phenotypes. The nutrient requirements and drug sensitivity of returned phenotypes are also being investigated for comparison with ground control values. Additionally, isolates of *T. terrestre* are being examined for changes in the ability to decompose human hair *in vitro*.

Ultraviolet Dosimetry

Two methods were employed to monitor the actual radiant energy penetrating selected optical components of the flight hardware. One of these methods involved Kodak High Resolution Film (Estar Thick Base) SO-343 which had been purged of oxygen and sensitized with dry nitrogen gas to decrease the rate of latent-image fading. This system was reliable over a range of 4×10^1 to 5.2×10^2 ergs cm^{-2} total energy with a peak wavelength of 254 nm. Postflight analyses indicated that the dosimeters received at least as much energy as had been expected from calculations based on data from the NASA established Solar Spectral Irradiance Standard (Thekaekara, 1971). The photographic film monitoring method proved to be a useful tool for measuring small amounts of UV irradiation in space.

Solar irradiation within the range of 4×10^4 ergs cm^{-2} to 4×10^5 ergs cm^{-2} was monitored by an adaptation of the Potassium Ferrioxalate Actinometry System described

by Wrighton and Witz (1972). Data collected from analysis of the contents of flight control and ground control cuvettes indicate that neither the simulated launch vibration nor the total space flight exerted a detectable change in preirradiated control systems. The ferrioxalate monitoring system, therefore, was shown to have the stability required for successful measurements made within the flight hardware. Analysis of inflight irradiated actinometry systems verified that the optical filter components of the Microbial Ecology Evaluation Device performed in a manner which allowed for critical evaluation of exposed biological test systems.

High Energy-Multicharged Particle Dosimetry

It was impossible, in the design of the flight hardware, to protect test systems from galactic irradiation. Therefore this factor had to be measured in order to better understand any observed biological effects. Data were obtained with two separate systems.

One set of measurements was obtained by strategically distributing 76 extruded thermoluminescent dosimeters composed of lithium fluoride wafers throughout the flight hardware. This distribution was used to allow dose determinations for each tier, for each of the six sides, and for the central volume of the closed assembly. Statistical analysis of the resulting data indicates that the various areas within the MEED received extremely uniform irradiation from the ionizing irradiation components of the space environment. Therefore, it is valid to omit this factor as a variable when comparing inflight test systems. The mean dose of all MEED thermoluminescent dosimeters (TLD) was 0.48 ± 0.02 rad with a range of 0.44 to 0.51 rad. Doses to crewmembers (from crew passive TLD measurements) were reported as ranging from 0.48 to 0.54 rad, with a mean of 0.51 ± 0.02 rad. The dose of 0.48 ± 0.02 rad represents a total absorption of 48 $\pm$ ergs of ionizing energy per gram of biological material within the MEED. This value was applicable to all samples within the flight hardware, including flight controls and UV irradiated samples.

The other set of galactic irradiation measurements was conducted in response to current concern for the effect of high energy-multicharged (HZE) particles on biological systems. A 2.5 x 3.8 cm container was provided within the flight hardware and ground control units to house four different types of dosimeters capable of recording these entities. Lexan dosimeters, identical to those employed in the crew personnel passive dosimeters, were used so that direct correlation could be made. Cellulose nitrate (CN) dosimeters were included in the MEED as well as in the Apollo Light Flash Moving Emulsion Detector (ALFMED) which was flown on Apollo 16, again allowing for direct comparisons. The other two detectors, Ilford G5 and silver chloride, were flown only in the MEED, but were of considerable value in establishing the HZE particle environment experienced by the flight hardware.

Both the Lexan and the cellulose nitrate (CN) detectors revealed track fluences (track cm^{-2}) of the HZE particles. Since the CN detector is more sensitive, it showed track fluences substantially higher than those found in Lexan. The sensitivity of the two detectors is such that the CN records particles with a Z (atomic number) greater than six, while Lexan records particles with a Z greater than ten. Comparison of Lexan and CN

track fluences found in the MEED flight hardware showed them to be somewhat lower than those found in either the ALFMED or the passive personnel dosimeters. These observations, along with the depressed TLD values presented above, imply that the MEED flight hardware had a somewhat greater average shielding as compared with either the ALFMED or the personnel passive detectors. Likewise, these data are slightly lower than those obtained from the TLD and CN detectors employed in the BIOSTACK flight hardware, which was stowed in the Command Module in an area of minimal shielding to ambient cosmic radiation.

Summary and Conclusions

This experiment system was designed to evaluate the effect of a particular space flight on the survival rate of nine different species. Although a reasonable variety of organisms (viruses, yeasts, filamentous fungi, bacteria, and an invertebrate) were tested under several different conditions, no statistically valid differences could be detected in the survival of flight samples when compared to corresponding ground-based controls. In general, these evaluations were based on multiple observations of from ten to thirty replicates of up to one million cells each. While the results of this experiment conflict with those of certain other space flight investigations, as noted in the Introduction, it must be observed that the conditions of a particular space flight cannot be exactly duplicated, and therefore results from different flights are not directly comparable.

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